AMRL-TR-66-187
(VOL. I)

INORGANIC FLUORIDE PROPELLANT OXIDIZERS

VOLUME I. THEIR EFFECTS UPON SEED GERMINATION AND PLANT GROWTH

DONALD J. REED, PhD
FRANK N. DOST, DVM
CHIH H. WANG, PhD

OREGON STATE UNIVERSITY

NOVEMBER 1967

AEROSPACE MEDICAL RESEARCH LABORATORIES
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Federal Government agencies and their contractors registered with Defense Documentation Center (DDC) should direct requests for copies of this report to:

DDC
Cameron Station
Alexandria, Virginia 22314

Non-DDC users may purchase copies of this report from:

Chief, Storage and Dissemination Section
Clearinghouse for Federal Scientific & Technical Information (CFSTI)
Sills Building
5285 Port Royal Road
Springfield, Virginia 22151

Organizations and individuals receiving reports via the Aerospace Medical Research Laboratories' automatic mailing lists should submit the addressograph plate stamp on the report envelope or refer to the code number when corresponding about change of address or cancellation.

Do not return this copy. Retain or destroy.

700 - March 1968 - CO455 - 27-587

AMRL-TR-66-187
(VOL. I)

INORGANIC FLUORIDE PROPELLANT OXIDIZERS

VOLUME I. THEIR EFFECTS UPON SEED GERMINATION AND PLANT GROWTH

DONALD J. REED, PhD
FRANK N. DOST, DVM
CHIH H. WANG, PhD

FOREWORD

This study was initiated by the Biomedical Laboratory of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The research was performed in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630204, "Environmental Pollution," under Contract No. AF 33(615)-1767 with the Radiation Center, Oregon State University, Corvallis, Oregon. Dr. C. H. Wang was the principal investigator for Oregon State University. Captain John A. Jurgiel and Sheldon A. London, Ph.D., were contract monitors for the Aerospace Medical Research Laboratories. Research was initiated in May, 1964 and completed in May, 1966.

The technical assistance of James Barnes, Jean Crecelius, Thurman Cooper, Moti Pinjani and Vernon Smith of Oregon State University has been very valuable to the conduct of this work.

This technical report has been reviewed and is approved.

WAYNE H. McCANDLESS
Technical Director
Biomedical Laboratory
Aerospace Medical Research Laboratories

ABSTRACT

Certain inorganic fluorides which are of interest as propellant oxidizers have been reviewed for their chemical, physical and toxicological properties. The compounds are nitrogen trifluoride, tetrafluorohydrazine, chlorine trifluoride, bromine pentafluoride, oxygen difluoride, hydrogen fluoride, and fluorine. Seeds and seedlings of bean, corn, pea, squash and sudan grass were exposed to air or water mixtures of these compounds. The seeds were more sensitive to solutions of hydrogen fluoride than solutions of sodium fluoride during germination. Exposure of dry seeds to a gaseous 100% NF_3 atmosphere for 1 to 8 hours caused inhibition of subsequent germination depending on the seed species. Exposure of seeds to a 1% N_2F_4 -air mixture for 1 hour had little effect upon their subsequent germination. However, exposure of seeds to a 10% N_2F_4 atmosphere for 1 hour completely inhibited germination of all five species of seeds. Exposure of dry seeds to less than 500 ppm of either bromine pentafluoride or chlorine trifluoride in air drastically reduced their subsequent germination even when the exposure time was for less than one hour. Exposures of plant seedlings to gaseous ClF_3 in air at 500 and 2,000 ppm for 5 minutes resulted in extensive destruction of the plants; bromine pentafluoride -air mixtures were even more damaging than ClF_3 atmospheres. Exposure of seedlings to N_2F_4 -air mixtures at 1,000 ppm for 30 minutes reduced growth rates of all plants; concentrations of N_2F_4 or NF_3 up to 10,000 ppm in air were required to cause visible damage of plant seedlings after a 30 to 60 minute exposure period. Plant injury was caused by irrigation of plant seedlings with solutions formed by the reaction of ClF_3 or BrF_5 with water. It was concluded that a major portion of the damage to plants by these inorganic fluorides was due either to their powerful oxidizing effects or to their ability to form fluoride ion. The exposed seeds and seedlings were analyzed for fluoride content. The equipment and methods used for these exposures are described.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

SECTION I

INTRODUCTION

The development of high-energy storable propellants as fuels for spacecraft propulsion presents a major challenge in the subsequent control of environmental pollution. Inorganic fluorides as propellant oxidizers are hazardous because of their reactivity and biochemical activity. The enormous quantities required for spacecraft propulsion cause these agents to be a part of our national concern for environmental pollution. The synthesis, transportation, storage, and utilization of these compounds are all potential pollution and hazard areas. The fluorine pollution problems of the aluminum industry are an example of the possible environmental pollution by inorganic fluorides in the spacecraft program.

The development of spacecraft technology has resulted in the Air Force having a major responsibility in propellant toxicology. The following report is the first part of a three-year contract at Oregon State University to study the pollution and environmental hazards of nitrogen trifluoride (NF_3), tetrafluorohydrazine (N_2F_4), chlorine trifluoride (ClF_3), bromine pentafluoride (BrF_5), and oxygen difluoride (OF_2). Laboratory experiments on the effects of these compounds on soils, water, vegetation, aquatic life, and certain microbial populations were therefore initiated. This research is performed to provide an index of toxicity and of the nature of potential hazards of these inorganic fluorides on our environment. Tolerance criteria for any propellant must be based upon its biochemical and pharmacological properties, pathways of environmental degradation, and its biological fate.

Little is known about the chemical properties of low concentrations of gaseous inorganic fluorides in air. We may well expect their chemistry to be different in low concentrations as compared to the concentrations used for investigating their reactivity with other compounds. For example, another class of toxic compounds, the halogenated acetylenes, have different chemical properties at low concentrations than at high concentrations in air. Mono- and dichloroacetylene, at high concentrations, explode spontaneously in air, yet recent studies have shown that dichloroacetylene is quite stable in air at concentrations of 10 ppm or less. Previous work by our laboratories and other workers has shown very similar properties for the pyrophoric compound, pentaborane-9. At high concentrations in air, this boron hydride will burn spontaneously; yet at low concentrations, it has a remarkable stability.

The inorganic fluorides under investigation are thermodynamically stable compounds. Their chemical properties as diluted gases in air or water have been investigated to only a limited extent. The toxicity of

several of these gases to various biological species has been studied under laboratory conditions. The extent to which environmental conditions are important in the toxicity of these inorganic fluorides to biological systems is still not known.

ClF_3, BrF_5 and OF_2 have chemical properties which result from their being mixed acid anhydrides, and the chlorine and bromine being in oxidized valence states. Therefore, these compounds have a powerful oxidizing capacity. They can also undergo hydrolysis during their interaction with biological materials and form fluoride ion and other intermediates. During these investigations, efforts were made to differentiate the effects of hydrogen fluoride and fluoride ion and the compound per se on the various biological species.

This report is divided into two main parts. The first part (sections II thru IV) is a literature review of the chemical and physical properties of the five inorganic fluorides of interest, fluorine and hydrogen fluoride. This part also contains a review of the known toxicological effects of these compounds. The second part (sections V thru VIII) is a major portion of the research which was carried out on the effects of four of these agents (NF_3, N_2F_4, ClF_3, and BrF_5) on seed germination and certain plants of economic importance.

SECTION II

A REVIEW OF THE PHYSICAL AND CHEMICAL PROPERTIES OF FIVE INORGANIC FLUORIDES, FLUORINE, AND HYDROGEN FLUORIDE

The following review of the properties of NF_3, N_2F_4, ClF_3, BrF_5, OF_2, F_2, and HF is limited to those properties thought to be most closely related to the toxic properties of these compounds. The physical constants given in Table I are supplemented by additional constants in the Appendix .

Nitrogen Trifluoride

Nitrogen trifluoride has been known since 1928 when Ruff, Fischer and Luft (ref.1) prepared it by the electrolysis of molten anhydrous ammonium bifluoride in an electrically-heated cell made of copper. Nitrogen trifluoride is a colorless gas at room temperature and condenses to a liquid at -129°[1].

The physical properties of NF_3, as shown in Table I and the Appendix were reviewed by Hoffman and Neville (ref.2) in 1962, and

[1]All temperature data are in degree centigrade units.

TABLE I

Physical Constants of Certain Inorganic Fluorides

Compound	Molecular Weight	Melting Point	Boiling Point	Critical Temperature	Critical Pressure in Atmospheres	Heat Capacity cal/mole/degree	Reference
Nitrogen Trifluoride	71	-206.79	-129.01	- 39.26	44.72	19.94	2, 3
Tetrafluoro-hydrazine	104	-162	- 73	.36	77	18.99	9
Chlorine Trifluoride	92.46	- 76.32	11.75	153.6		15.2	24, 25, 26
Bromine Pentafluoride	174.92	- 62.5	40.3	197		24.49	24, 32
Oxygen Difluoride	54	-223.8	-145.3	- 58.0	48.9	10.35	34
Fluorine	38	-219.62	-188.14	-129	55		51, 52, 53
Hydrogen Fluoride	20.01	- 83	19.5	23			54

All temperatures are in degree centigrade units.

and Pankratov (ref.3) in 1963. Microwave spectra (ref.4) are consistent with a pyramidal structure with a F-N-F angle of 102° 9' and a NF bond distance of 1.371 Å. These parameters are in reasonable agreement with those determined by electron diffraction (ref.5). Stark splittings of microwave lines yield dipole moments of 0.234 ± 0.004 or 0.235 ± 0.007 Debye units (ref.6).

The bond dissociation energies (ref.7) are as follows: $D(NF_2\text{-}F) = 57.1 \pm 2.5$, and the mean of D(NF-F) and D(N-F) = 71 kcal/mole.

The chemical properties of NF_3 have been investigated and NF_3 is surprisingly unreactive. Its principal characteristic is that of a strong oxidizing agent. It does not react with dry glass and is unaffected by dilute basic solutions or dilute sulfuric acid. It is somewhat soluble without immediate reaction in water. NF_3 will react with water vapor by sparking. The products are HF, NO and NO_2. An electrical discharge will cause mixtures of NF_3 to react explosively with many gaseous reducing agents (H_2S, NH_3, H_2, CH_4, C_2H_4, CO).

Hurst and Khayat (ref.8), in 1964, examined the reaction of NF_3 with aqueous solutions of various acids, bases and salts. The reactions of NF_3 with several nucleophiles ($HClO_4$, HNO_3, H_2SO_4, HCl, NaCl, HBr, NaBr, NaOH, NaI, HI, and $Na_2S_2O_3$) were carried out at 100 and 133° for 159 to 235 hours. The greatest degree of reaction of NF_3 with these compounds occurred in the presence of 4 N HCl (63.6% NF_3 reacted in 160 hours) and 10.5 N HI (71.8% NF_3 reacted in 160 hours). In the presence of 0.5 M sodium hydroxide at 100° for 160 hours, 38.2% of the NF_3 was destroyed. Various reaction products were identified from these reaction mixtures. Many metals will react with NF_3 at elevated temperatures to produce N_2F_4. These metals include stainless steel, copper, arsenic, antimony and bismuth (ref.9).

Tetrafluorohydrazine

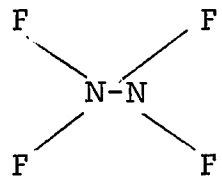
Colburn and Kennedy (refs.10, 9) first prepared tetrafluorohydrazine by the thermal reaction of nitrogen trifluoride with various metals such as stainless steel, copper, arsenic, antimony, and bismuth, according to the equation

$$2\ NF_3 + 2M \longrightarrow N_2F_4 + 2MF$$

Since the first synthesis in 1958 several other methods have been described (ref.2). Tetrafluorohydrazine is a colorless gas possessing a musty odor. It condenses at -73° to a colorless liquid (refs.11, 12).

Most of the physical constants listed in Table I were determined by Colburn and Kennedy (ref.9). Additional characterizations of N_2F_4 by these workers included the determination of the infrared spectrum, the

mass spectrum, and the fluorine-19 NMR spectrum. The microwave spectrum of tetrafluorohydrazine has been investigated (ref.13) and the observed rotational constants found to be consistent with a hydrazine-like model.



Johnson and Colburn (ref.14) have reported that tetrafluorohydrazine exists in a dissociative equilibrium with the difluoramino free radical

$$N_2F_4 \rightleftharpoons 2 \cdot NF_2$$

The difluoramino radical is quite stable and is capable of existing indefinitely in the free state. This behavior is very similar to that shown by nitrogen dioxide, which exists in equilibrium with dinitrogen tetroxide

$$N_2O_4 \rightleftharpoons 2 \cdot NO_2$$

The behavior of these two free radicals, $\cdot NO_2$ and $\cdot NF_2$, is quite different from that of the amino free radical ($\cdot NH_2$). Upon heating to high temperatures hydrazine (N_2H_4) is thought to dissociate into two amino free radicals.

$$N_2H_4 \rightleftharpoons 2 \cdot NH_2$$

However, amino free radicals are extremely reactive and the end products of the hydrazine dissociation are in general ammonia, nitrogen, and hydrogen.

Colburn states that the difference in the behavior of the difluoramino -and the amino-free radicals lies in the difference in the order of bond strength in ammonia and that in nitrogen trifluoride. The dissociation equilibrium of N_2F_4 into difluoramino radicals is readily investigated because the radicals absorb light in the ultraviolet region which permits measurement of the formation of even a fraction of 1% of the radicals. EPR spectra were obtained which indicated that the radicals as well as the tetrafluorohydrazine are relatively unreactive toward glass at temperatures up to those at which the dissociation to N_2F_4 is almost complete (ref.14). It can be expected that the difluoramino radicals may undergo some of organic reactions <u>in vivo</u> which have been described below.

Frazer (refs. 15, 16) and Petry and Freeman (ref.17) have reported the reactions of difluoramino radicals with radicals generated from diketones,

methyl and ethyl iodide, azo intermediates and aldehydes. Thus one can conclude that the difluoramino radical will combine with free radicals which are generated in its presence.

A limited number of studies have been reported on the reactions of N_2F_4 with air atmospheres. Stevenson[1] (personal communication, 1966) examined the stability of N_2F_4 in air and concluded that its stability was related to the moisture content of air and the metal surface of the chamber used to contain the N_2F_4-air mixtures.

Beach (ref.18) described the formation of NOF from N_2F_4 in the presence of oxygen. The reaction was carried out in a brass-bodied infrared cell equipped with NaCl windows.

Hurst and Khayat (ref.8) examined the reactions of N_2F_4 with water and aqueous solutions of NaOH and HCl. The reaction with NaOH produced mainly nitrous oxide and nitrite with a trace of nitrogen. The reaction of N_2F_4 with water at 80° appeared to proceed according to the equation

$$N_2F_4 + 2\ H_2O \xrightarrow{80^\circ} 2\ NO + 4\ HF$$

These authors suggest that the hydrolysis of N_2F_4 may proceed much differently if trace impurities are present, such as

$$N_2F_4 + 2\ NO_2\ (\text{trace}) \longrightarrow 4\ NOF$$
$$4\ NOF \xrightarrow{H_2O} 2\ NO + 2\ NO_2, \text{ etc.}$$

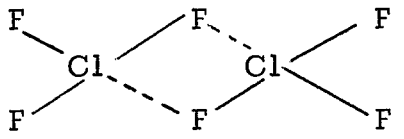
Chlorine Trifluoride

Chlorine trifluoride is a nearly colorless gas at atmospheric temperature and pressure. It boils at 11.75° and melts at -76.32°. It has a somewhat sweet odor and is highly irritating to the eye and respiratory tract even at low concentrations. Physical constants for ClF_3 are listed in Table I.

The infrared and Raman spectra of chlorine trifluoride have been reported by Jones, Parkinson and Murray (ref.19).

The determination of the dipole moment (ref.20), X-ray diffraction studies (ref.21), microwave studies (refs.22, 23) all indicate that the molecular structure of liquid chlorine trifluoride is planar and in the nature of a symmetric dimer. Some dimer association exists in the vapor state of ClF_3.

[1]Dr. Kenyon Stevenson, Head, Chemical Engineering Section, Redstone Arsenal Research Division, Rohm & Haas Co. Huntsville, Alabama.



At ordinary temperatures, chlorine trifluoride reacts with every element with the exception of nitrogen and possibly platinum and palladium. Chlorine trifluoride is the most chemically-reactive compound among the known interhalogen fluorides. Of considerable importance in the handling of ClF_3 is the fact that it is a vigorous incendiary causing immediate ignition of organic substances; it reacts violently with water or ice and ignites many metals at elevated temperatures. It is a vigorous fluoridating agent, having essentially the same ability as elementary fluorine to raise metals to their highest valence state. In a few cases, under control conditions, fluoride and chlorine may be introduced into organic molecules with chlorine trifluoride. In the reaction of chlorine trifluoride with water, the compounds formed are numerous and their relative proportion is a function of the conditions under which chlorine trifluoride comes into contact with water. Possible reactions and the products which may be formed are as follows:

$$4\ ClF_3 + 3H_2O \longrightarrow 6HF + 3OF_2 + 2Cl_2$$

$$2\ ClF_3 + 2H_2O \longrightarrow 4HF + ClO_2F + ClF$$

The following secondary reactions may also occur:

$$ClO_2 \longrightarrow 1/2\ Cl_2 + O_2$$

$$2\ ClO_2 + ClF_3 \longrightarrow 2\ ClO_2F + ClF.$$

Thus, the reaction of ClF_3 with water appears to be very complex but has not been investigated fully. Reviews on material compatibility of ClF_3 and its chemical properties are numerous (refs. 24, 25, 26, 27, 28).

Bromine Pentafluoride

Bromine pentafluoride is a pale yellow liquid which solidifies at -62.5° and boils at 40.3° (refs. 29, 30). Molecular weight determinations indicate that there is no association in the vapor state (ref. 31).

The physical properties of BrF_5 are shown in Table I (refs 24, 32).

McDowell and Asprey (ref.33) reported the infrared spectrum of bromine pentafluoride. The spectrum confirms a square pyramidal structure. The main infrared absorption peaks occur at 684, 644, 584, 415, 370 and 245 cm^{-1}. Bromine pentafluoride probably reacts with every known

element with the possible exception of nitrogen and oxygen. Even under ambient conditions, it vigorously attacks organic compounds. Thus, bromine pentafluoride is an extremely hazardous material to handle.

Ruff and Menzel (ref.29) found that lithium powder, barium, zinc, mercury, molybdenum, tungsten, iron, cobalt, arsenic, and antimony react immediately with bromine pentafluoride at room temperature. Temperatures of 300° or above were necessary for the reaction of bromine pentafluoride with copper, cadmium, powdered titanium and platinum. Moderate warming was found necessary to start the reaction with silica and glass. Bromine pentafluoride vigorously reacts with organic compounds. The reaction is usually accompanied by flame and often approaches explosive violence. There is no recorded data of a desired organic fluoride being made by the use of bromine pentafluoride. All of the precautions necessary for the handling of chlorine trifluoride are apparently necessary for handling bromine pentafluoride. The relatively high boiling point of bromine pentafluoride can result in the filling of distribution lines and valves with liquid bromine pentafluoride during sample transfer if special precautions are not taken to keep the entire manifold system heated to 40° to 50°. Condensation of BrF_5 in control valves can cause increased vapor pressure of BrF_5 in the distribution system, and ignition is likely to occur at some point in the system, particularly a valve.

Oxygen Difluoride

Oxygen difluoride is a colorless gas at atmospheric temperature and pressure, condensing to a pale yellow liquid at -145° (ref.34). Melting point of the solid is -223.8° (ref.35). The physical constants for OF_2 are given in Table I. OF_2 has a very unpleasant, irritating odor. However, maximum precautionary measures are necessary to prevent the inhalation of OF_2 because the olfactory senses quickly become somewhat insensitive to this compound.

In 1927, Lebeau and Damiens first synthesized OF_2 by combining fluorine and oxygen, which occurred while preparing fluorine in an electrolytic cell at about 100°, with molten (slightly moist) KF-HF as the electrolyte (ref.36). Later, the same workers found that the reaction of F_2 with aqueous NaOH (ref.37) also forms OF_2. This reaction is generally employed for the preparation of OF_2. The solubility of OF_2 in water obeys Henry's law. About 6.8 cc of gaseous OF_2 can be dissolved in 100 ml of cold water. The velocity of the reaction between OF_2 and water has been studied (ref.38).

Electron diffraction (ref.39) and infrared spectrum (ref.40) studies indicate that the F-O-F angle of the OF_2 molecule is about 100°. The bond

is estimated to be approximately 6% ionic in nature.

Oxygen difluoride is a powerful oxidizing agent, but it is generally considered to be much less reactive than fluorine. It is a relatively stable compound in that it does not detonate by sparking, but it does begin to decompose thermally at approximately 250°.

Pure dry oxygen difluoride is relatively stable at room temperature. It can be kept in glass vessels at room temperature for a long period without noticeable decomposition (ref.41).

Oxygen difluoride is slightly decomposed by light. Mainly short-wavelength light ($\lambda < 3000$ Å, predominantly $\lambda < 2500$ Å) is absorbed, and OF_2 decomposes to O_2 and F_2 (ref. 42).

Chemically, OF_2 is a powerful oxidizer. In reactions with solids and upon warming, OF_2 reacts as a fluorinating agent, while in aqueous solutions the addition of oxygen is the main reaction (refs. 43, 44, 45).

In aqueous solutions of HCl, HBr, and HI, oxygen difluoride reacts quantitatively, liberating free halogens during the formation of HF.

$$OF_2 + 4HCl \longrightarrow 2Cl_2 + 2HF + H_2O$$

Halogens are displaced from their salts by OF_2. In the reaction with aqueous NH_3, nitrogen is liberated, and the formation of HNO_3 takes place. Reactions with aqueous solutions of KOH, NaOH and $Ca(OH)_2$ cause absorption of OF_2 and liberation of gaseous oxygen and destruction of all oxidizing compounds in solution. From an aqueous solution of H_2S, oxygen difluoride precipitates colloidal sulfur. Many aqueous solutions of salts react with OF_2 causing oxidation of the metal ions to a higher valence state. Gaseous OF_2 may be safely mixed with H_2, CH_4, and CO but the mixtures explode violently when a spark is created. Oxygen difluoride can be reacted with certain organic compounds (refs. 46, 47, 48). Benzene and paraffinic compounds readily absorb gaseous OF_2. Methanol and ethanol react slowly with OF_2 at room temperature. When OF_2 is dissolved in CCl_4 at room temperature one-half of the dissolved OF_2 will react in 48 hours. The reaction of OF_2 with metals, nonmetallic solid substances, halogens, and with many gases has been reviewed by A. G. Streng (ref. 49).

Rhein and Cady (ref.50) have studied many of the reactions of OF_2 with numerous other compounds.

Fluorine

Certain of the physical properties of fluorine are given in Table I (refs. 51, 52, 53). At ordinary temperatures fluorine is a pale yellow gas; it condenses to a yellow liquid at -188.14° and this solidifies to a yellow solid at about -218°, the color of the yellow solid changing to white. Electron diffraction pattern of the fluorine molecule has shown the F-F distance to be 1.44 Å.

Fluorine is the most powerful oxidizing agent known and is therefore the most electro-negative element. Solid fluorine reacts extensively with liquid hydrogen, even at -252°. Boron and silicon burn in fluorine, forming boron trifluoride and silicon tetrafluoride. Amorphous carbon reacts with fluorine to yield carbon tetrafluoride together with small quantities of other fluorocarbons. Most metals are attacked by fluorine at ordinary temperature. Fortunately, however, reaction of fluorine with many metals is slow at room temperature and often results in the formation of a metal fluoride film on the surface of the metal. This film greatly retards further reaction in the case of certain metals, such as brass, iron, aluminum, magnesium and copper. Careful passivation of these metals allows them to be quite satisfactory for handling fluorine at room temperature. Fluorine reacts with water producing hydrogen fluoride and oxygen. Small amounts of ozone, hydrogen peroxide and oxygen difluoride are produced during the reaction. However, the ozone formed may be destroyed by the heat of the reaction. Oxygen difluoride is produced by the reaction between fluorine and basic solutions. Fluorine reacts with organic compounds in a vigorous and often explosive fashion unless special handling techniques are utilized. Controlled interaction of fluorine and organic compounds has resulted in the synthesis of the remarkable fluorocarbon compounds.

Hydrogen Fluoride

Anhydrous hydrogen fluoride is a colorless liquid freezing at -83° and boiling at 19.5° (ref.54). The density of hydrogen fluoride at 88° and 740 mm corresponds with the formula HF. Between 28° and 38° and at low pressures the density corresponds to a formula of $(HF)_2$ and to more highly associated products of HF at higher pressures. Electron diffraction patterns of the vapors indicate the presence of polymers of HF with aggregation up to H_5F_5. The abnormally high boiling point for

hydrogen fluoride can be accounted for by the polymeric state of hydrogen fluoride in the vapor state. An aqueous solution of hydrogen fluoride is a weak acid and forms a constant boiling mixture of minimum vapor pressure. The solution which boils at 120° contains 36% hydrogen fluoride.

Unless completely dry, hydrogen fluoride attacks the alkali and alkaline earth elements at room temperature. Aqueous solutions of HF attack most metals, evolving hydrogen. Hydrogen fluoride and silica form silicon tetrafluoride. Silica and aqueous hydrogen fluoride yield fluorosilicic acid.

SECTION III

TOXICITY OF INORGANIC FLUORIDES

Efforts have been made to review all published reports on the toxicity of NF_3, N_2F_4, ClF_3, BrF_5, and OF_2. An exhaustive search was not attempted for all reports on the biological effects of fluorine, hydrogen fluoride, and fluoride ion. However, it is reasonable to assume that the reports cited do represent current knowledge concerning the toxic effects of these agents.

Toxicity of Nitrogen Trifluoride

Only a few reports have been published on the toxicological properties of nitrogen trifluoride. Ruff (ref.55), in 1931, reported that although the mono and difluoro derivatives of ammonia (NH_2F and NHF_2) were quite toxic, nitrogen trifluoride was rather low in acute toxicity when inhaled. He also reported that the compound produced methemoglobinemia.

Torkelson et al. (ref.56), in 1962, reported studies on the toxicity of NF_3 to rats. They concluded that a single exposure of rats to concentrations of NF_3 in excess of 1,000 ppm caused methemoglobinemia, and that a concentration of 2,500 ppm caused death after 4 hours of exposure. They also reported data obtained from experiments in which rats of both sexes were given repeated 7-hour daily exposures to 100 ppm for 4 1/2 months. These exposures resulted in slight to moderate pathological changes in the livers and kidneys of both sexes. An increase was also found in the average weight of the liver, kidneys, and spleen of the male rats. No evidence of fluorosis of the teeth or deposition of fluoride in the teeth and bone was observed, although a very slight increase in total fluorine in the urine was detected.

Toxicity of Tetrafluorohyrazine

In 1962, Clayton (ref.57) reported a 4-hour, 50% lethal concentration (LC_{50}) for N_2F_4 of 50 ppm when rats were used as test animals. The principal effect of exposure to N_2F_4 at concentrations which ranged from 25 to 90 ppm air was on the lungs, kidneys, and the hematopoietic system. Signs of slight nasal and mouth irritation were noted with exposures of 10 ppm for 4 hours. However, a glass syringe pump was used to disperse N_2F_4 into an exposure chamber and consequently, at the low concentrations of N_2F_4 utilized, it is doubtful whether N_2F_4 remained intact in the exposure chamber or even the syringe.

In 1964, Carson and Wilinski (ref.58) extended the work on the toxicity of N_2F_4. These workers indicated problems in exposure of animals to N_2F_4 due to the reactivity of N_2F_4 with air. To carry out exposures, N_2F_4 gas was metered from its cylinder through a stainless steel line and a manometer into a mixing bowl attached to either a 125- or 400-liter dynamic flow gassing chamber. The chamber atmosphere was analyzed by drawing aliquots of the atmospheres through absorbers connected in series, each containing 10 ml of 10% alcoholic potassium hydroxide. Aliquots of the solution were subsequently analyzed for fluoride content. Concentrations of N_2F_4 as ppm were calculated from the amount of airborne fluoride in the chamber. Even while using this method of analysis, the authors reported that N_2F_4 was found to be unstable in the presence of air for more than a few minutes. They reported no histopathological changes in animals exposed to lethal and sublethal concentrations of N_2F_4 in 15- and 60-minute exposures. Some pathological changes were observed in the lungs, spleens, and livers of animals exposed to lethal concentrations of N_2F_4 for 4-hours. The toxic signs were cyanosis, hematologic changes, and eye and nasal irritation. The reported LC_{50} values for rats from single 15-, 60-, and 240-minute exposures to N_2F_4 were 9,650, 950, and 120 ppm, respectively. The 60-minute LC_{50} value for guinea pigs was 900 ppm. Carson and Wilinski concluded that in both their experiments and those of Clayton, the toxicity of N_2F_4 was due, in part, in at least a portion of their experiments, to breakdown products of N_2F_4. They concluded that breakdown products included nitric oxide, nitrosylfluoride, nitrogen trifluoride, and hydrogen fluoride. They reported that an additional toxic hazard of N_2F_4 was methemoglobinemia. However, in all their experiments even at lethal concentrations of N_2F_4, methemoglobin content of exposed animals did not exceed 20% of the total hemoglobin.

Stevenson (private communication, 1964) carried out a limited number of experiments to establish an LC_{50} value for N_2F_4 for male Wistar rats. With 4-hour exposures, the LC_{50} value was 50 ppm. For exposure times of 30 and 60 minutes, the values were 1,200 and 400 ppm, respectively. Stevenson's investigations were carried out in a dynamic-flow stainless

steel exposure chamber which was monitored by infrared absorption. In this manner, it was possible to demonstrate that the atmospheres of N_2F_4 did not contain the breakdown products suggested by Carson and Wilinski to be present during their experiments.

Toxicity of Chlorine Trifluoride

Chlorine trifluoride is a highly toxic material and its toxicity is approximately the same order of magnitude of that of anhydrous hydrogen fluoride. Thus, by analogy, a maximum allowable concentration for an 8-hour day of only 3 ppm by volume in air has been suggested. Concentrations of 50 ppm or more may be fatal in 30 minutes to 2 hours. At concentrations of 100 ppm, toxicity symptoms were noticed after 3 minutes in experimental animals; at 500 ppm, symptoms appeared at once; the symptoms being gasping for breath, cloudiness of the cornea, lacrimation, swelling of eyes and eyelids, severe salivation and acute distress. Death was preceded by convulsions in many cases. In practice, fatal concentrations would be so irritating to the eyes and respiratory tract as to make the area intolerable. The odor threshold is quite low though not known quantitatively. The toxic symptoms appear more slowly in lower concentrations in air, but in all cases where the maximal allowable concentration is exceeded, the symptoms of eye and respiratory irritation appear (ref.59).

In 1953, Horn (ref.60) reported a study on the toxicity of chlorine trifluoride. In his experiments, two dogs and 20 rats were exposed to an average concentration of 1.17 ppm chlorine trifluoride for a total period of 6 months on a 6-hour-per-day, 5 day-per-week basis. An additional two dogs and 20 rats, housed in the same room, served as controls. Signs of toxicity in the dogs consisted of coughing, sneezing, rhinorrhea, lacrimation, salivation, a "panting" type of respiration, and occasionally the coughing up of a frothy fluid. After about 2 months, both dogs had recurrent bouts of pneumonia. Signs were not so pronounced in the rats, but after several weeks they had a blood-tinged discharge about their nose and eyes. Pathological findings, both gross and microscopic, indicated pulmonary irritation in the exposed animals. Of the experimental animals which died, there was evidence of pulmonary abscesses and bronchopneumonia.

Toxicity of Bromine Pentafluoride

There are no reported data on the toxicity of bromine pentafluoride. However, it would be expected to display toxicological properties similar to those of chlorine trifluoride.

Toxicity of Oxygen Difluoride

Though toxicity of OF_2 has been studied only to a limited extent, the available literature reveals that this compound is highly toxic even at very low concentrations--concentrations at which the odor of the gas may be detectable. LaBelle (ref.61) investigated the effects of OF_2 exposure by inhalation on mice, rats, and guinea pigs. A single exposure of these animals to 5 ppm of OF_2 caused 100% mortality in 7 hours or less. When the concentration of OF_2 was 10 ppm, all animals died in 4 1/2 hours or less. In 1961 Cianko (ref.62) used rats and guinea pigs to examine the toxicity of OF_2. He concluded that the lethal concentration of OF_2 was in the range of 50 ppm. Cianko reported, as had LaBelle, that the primary damage was in the pulmonary tract, resulting in hemorrhage and edema, but that some systemic damage was observed.

Lester (refs. 63, 64) carried out an extensive pathological examination of rats exposed to OF_2. He concluded that the CT product (C as ppm and T as minutes) may be estimated for a 50% mortality to be about 100. He found, without exception, widespread injury to the lungs. While the corrosive damage to the lungs was extensive, severe respiratory distress was observed only immediately prior to death. Of considerable importance was the observation that the first signs of acute pathology took many hours to become evident, in some cases more than 7 hours, and that no behavioral signs of ill effects appeared during exposure of the rats. He observed that the odor of oxygen difluoride, though unique and first detectable at 0.1 ppm, is not at all displeasing, and that the probability of rapid adaptation to the odor is very high.

Knapp (private communication, 1964)[1] has concluded from the evidence available that the pulmonary damage by OF_2 is due primarily to the relatively slow hydrolysis of OF_2 to HF. The formation of HF in alveolar capillaries of the lung causes massive destruction of the cells while inhalation of gaseous HF causes damage to only the upper bronchial passages, presumably due to the rapid absorption of HF.

Effects of Hydrogen Fluoride Administered by Inhalation

Several reports describe the early work on the toxicity to animals of hydrogen fluoride inhalation (refs. 65, 66, 67).

In 1934, Machle et al. (ref.65) described morphologic changes in animals after single, brief exposures to HF vapor. They observed visceral

[1] Dr. W. A. Knapp, General Chemical Division, Allied Chemical Corp, Morristown, New Jersey.

congestion, myocardial necrosis and edema, pulmonary hemorrhage, edema and emphysema, hepatic necrosis and hemorrhage, and renal tubular necrosis. In rabbits and guinea pigs subjected to lethal concentrations of HF vapor, Stokinger (ref.66) found 100% mortality in rats and mice which had inhaled 25 mg/m^3 of hydrogen fluoride for 166 hours. The same exposure of guinea pigs, rabbits and dogs showed no mortality. No deaths in any species were observed at 7.2 mg/m^3 of hydrogen fluoride. At much higher concentrations (1,000 to 10,000 mg/m^3), exposures up to 41 hours were almost certainly lethal.

In 1962, Rosenholtz (ref.67) reported that distinctive hepatic and renal lesions resulted from the exposures of rats to lethal dosages of hydrogen fluoride. Severe local effects of contact were also noted. The systemic effects of exposure represented a form of fluoride ion poisoning. The systemic lesions include renal tubular necrosis and hepatocellular lobular change.

Effects of Fluorides on Biological Systems

The reaction of oxidizing inorganic fluorides with biological systems can result in the formation of fluoride ion. Investigations to date do not delineate entirely between the toxic effects of the inorganic fluoride oxidizers per se and their breakdown products. Therefore, the effects of fluoride will be reviewed briefly.

Reviews on fluoride and its effects upon biological species are very numerous. Only a few will be mentioned in this report. In 1933, McClure (ref.68) reviewed the physiological effects of fluoride. In 1965, Hodge and Smith (ref. 69) reviewed the literature on fluoride intoxication. In 1960, Princi reviewed the effects on man of the absorption of fluoride (ref. 70). In 1963, Waldbott (ref. 71) reviewed acute fluoride intoxication of both man and animals by various fluoride-containing organic and inorganic compounds. Largent (ref. 72) has reviewed fluorosis and the health aspects of fluorine compounds. A symposium on the physiological and hygienic aspects of the absorption of inorganic fluorides has been published (ref. 73). Hodge and Smith have written a book on the biological properties of inorganic fluorides (ref. 74). Effects of fluorides on plants have been reviewed (refs. 75, 76, 77). Simonin and Pierron (ref.78) have reported the lethal doses of some 30 different inorganic fluoride salts in adult guinea pigs.

In addition to review articles of the biological effects of fluorides, a few specific references are cited which described lethality data for fluoride ion. Leone (ref. 79), in 1965, described the acute and chronic toxic effects of sodium fluoride in dogs and mice. The mean acute lethal dose of sodium fluoride in unanesthetized dogs (infused to death by continuous

intravenous infusions at the rate of 5.4 mg of fluoride ion/min) was 36.0 ± 0.5 mg/kg. The principal effects were progressive depression of blood pressure, heart rate and central nervous system, with vomiting and defecation, all occurring with the administration of approximately 20 mg/kg. At a mean dose of 30.6 mg/kg, there was a depression of respiratory rate and a conversion to atrioventricular nodal or ventricular rhythm, with terminal ventricular fibrillation or asystole. An approximate LD_{50} was estimated to be 20 mg/kg. In the fatal cases, death occurred in 1 to 36 hours. Single doses up to 3,100 mg/kg by mouth in dogs produced only vomiting, defecation, and transient moderate depression. The intravenous LD_{50} value was 23.0 ± 0.9 mg/kg and the oral LD_{50}, 46.0 ± 1.6 mg/kg. A slight drop in serum calcium followed the infusion of fluoride in most dogs in which serum calcium was determined.

In 1960, Princi (ref.70) described the effects on man of the absorption of fluoride. He pointed out that most cases of acute fluoride intoxication have resulted from the ingestion of large doses of fluoride compounds. The effects of these substances on the gastro-enteric tract have been well described. The severity of the symptoms is in direct proportion to the irritating properties and to the amount of the compound that has been ingested. Thus vomiting may be clear or hemorrhagic; diffuse abdominal cramps are usually present, and diarrhea is a common occurrence. With sufficiently large doses of the fluoride compound and effective absorption, involvement of the central nervous system can be expected. Tonic and clonic convulsions may occur and may persist. Twitching of muscle groups is not uncommon. Thirst, diaphoresis, and salivation have been reported commonly. Although it has been suggested that fluoride compounds may produce a paralytic reaction, the immediate effect is probably one of intense stimulation of the central nervous system with a resulting exhaustion of the nervous pathways.

In 1965, Lu et al. (ref.80) investigated the acute toxicity of sodium fluoride for Rhesus monkeys, squirrel monkeys and albino rats. Intravenous injections (1 mg/kg/min) of sodium fluoride into Rhesus monkeys caused a fall in blood pressure, bradycardia and depression of the central nervous system. There was marked reduction in serum calcium (9.63 ± 0.266 mg/100 ml to 3.55 ± 0.780), but the magnesium level was only slightly lower (1.68 ± 0.112 to 1.40 ± 0.123 mg/100 ml) after a lethal I.V. dose (65.8 ± 6.32 mg/kg) of fluoride administered as sodium fluoride.

Carlson et al. (ref.81) reported on the distribution of radiofluoride, F^{18}, in whole blood. In vitro and in vivo experiments indicated that 75% of the F^{18} was in the plasma and the remainder in erythrocytes and that the distribution was influenced by carbon dioxide tension associated with the pH of whole blood. Less than 5% of the radiofluoride plasma was bound to plasma solutes under physiological conditions. F^{18} binding by bovine plasma albumin was increased by increasing the calcium ion concentration and also by increasing the pH of the solution.

Recently, Call et al. (ref. 82) reported studies on the chronic exposure of man to fluoride. They state that the presence of elevated concentrations of fluoride in the atmosphere has caused an increase in the fluoride content in plants in certain areas in Utah. Long-term ingestion of such forage by animals has produced some changes characteristic of fluorosis. Call et al. autopsied 127 human bodies and studied them for gross, histological, and chemical evidence of fluoride intoxication. Eighty-eight of these deceased persons came from geographic areas known to have had elevated fluoride levels in the atmosphere and forage. Analyses for fluoride, calcium, phosphorus, and ash were made, and the highest fluoride levels were found in adults showing the end-stage kidney of bilateral pyelonephritis and polycystic disease. The highest fluoride levels found, in subjects with the most severe kidney disease, were within the normal range, and no disease associated with fluoride was evident. From their data, they concluded that the levels of fluoride to which Utah residents in the area studied had been exposed were not responsible for gross or histological changes in soft tissues or bones.

Taylor et al. (ref. 83) described the acute injury of the rat kidney after single large doses of sodium fluoride. The sodium fluoride was administered in the drinking water of weanling rats. With levels of 150 to 250 ppm fluoride administered in the water, 30 to 40% of the surviving rats showed a renal lesion consisting of dilatation of the tubules of the corticomedullary junction.

Inhibition of Enzymes

The work reported on the in vitro inhibition of enzymes indicates that the biochemical effects of fluoride on biological species are complex. Inhibition of the enzyme enolase (ref. 84) is perhaps the best known of the effects of fluorides on enzymes in vitro. The degree of inhibition is dependent upon the magnesium and phosphate concentrations as well as upon the fluoride concentrations. It has been postulated that enolase is inhibited by the formation of a magnesium fluorophosphate complex but fluorophosphate itself is not inhibitory (ref. 85). The hypothetical complex may be formed on the enzyme to cause inhibition. Magnesium-activated phosphatase and pyrophosphatase (ref. 86), manganese or magnesium-activated phosphoglucomutase (ref. 87) and iron-containing succinic dehydrogenase (ref. 88) are fluoride sensitive, and again inhibition may be the result of the formation of the metal fluorophosphate complex. Fluoride is a competitive inhibitor of human acid phosphomonesterase (ref. 89). Inhibition of human prostatic acid phosphomonesterase is also a function of fluoride concentration (ref. 90). Fluoride at 10^{-4} M concentration causes 80% inhibition of the purified enzyme while 10^{-5} M resulted in 7% inhibition. Fluoride has been demonstrated to combine with the ferric iron in the heme groups of methemoglobin, peroxidase, catalase and cytochrome oxidase (ref. 91).

The effect of metal and phosphate concentration indicates that the nutrient status of any species may affect its sensitivity to fluoride toxicity (refs.92, 93). Thus, the concentration and distribution of fluoride within the cell may determine not only the degree to which metabolism is affected but also the type of metabolic effect.

Effects on Metabolism

In addition to a relatively specific effect on certain enzymes, fluoride may exert a general effect on metabolism by the formation of metal fluoride complexes. The altered nutritional status of cells could result in subsequent decreased activity of their metal-requiring enzymes, or even a weakening of cellular integrity which is dependent upon calcium or other metals (ref.94). The respiratory activity of cells based upon glucose as a substrate may depend upon the pentose phosphate, glycolytic or the glucuronate-xylulose pathways. The production of $^{14}CO_2$ from specifically-labeled glucose has been used to assess the relative importance of these pathways for carbohydrate metabolism. For a discussion on pathway estimation of glucose metabolism, see references 95, 96 and 97.

In fluoride-fed rats (ref.98) the effect of dietary fluoride ingestion (450 ppm fluoride) on the relative importance of the pentose phosphate and Embden-Meyerhof pathways of glucose catabolism was related to the level of liver glucose 6-phosphate dehydrogenase. The level of this enzyme was decreased approximately 50% by fluoride. However, the decrease in enzyme activity in fluoride-fed rats was a consequence of a direct effect of fluoride on the pattern of food intake.

Aqueous solutions of sodium or potassium fluoride inhibited the exchange of such gases as oxygen (O_2 uptake) and carbon dioxide (CO_2 release) in isolated plant tissues. The reversal of this inhibition by the addition of pyruvate but not by the addition of glucose or sucrose in spinach leaves (ref.99), barley roots (ref.100), and Avena coleoptile sections (ref.101) indicates that the site of fluoride inhibition of respiration in these plant tissues was in the glycolytic pathway. As plant tissue matures or increases in its physiological age, glucose catabolism appears to shift from glycolysis to the pentose phosphate pathway. In wheat leaves, decreased sensitivity of oxygen uptake or $^{14}CO_2$ production to fluoride inhibition was observed (ref.102). In some plant tissues, an increased exchange of gases is elicited by fluoride. The stimulation of O_2 consumption occurs in root tips of Lens culinaris (ref.103), leaf discs of grape and apricot (ref.104), and pea epicotyl sections (ref.105) at fluoride concentrations lower than those producing inhibition of oxygen uptake. The similarity of the effects of 2,4-dinitrophenol and carbon monoxide on respiration and cellular activity (ref.106) to the stimulation of O_2 uptake and the inhibition of endergonic processes involved in biosynthesis by fluoride suggests that fluoride may be acting as an uncoupling agent and may be inhibiting phosphory-

lation. In general, experiments with isolated plant tissues show that a variety of metabolic pathways may be affected by fluoride and that the presence or type of effect depends on which pathways are operative. Moreover, many factors such as age and the level of endogenous metabolites or growth hormone can alter the metabolism of the plant and, thereby, alter its fluoride sensitivity.

Effect on Metabolism as Related to Leaf Necrosis

Some of the metabolic responses observed in isolated tissues exposed to aqueous solutions of fluoride have also been observed in intact plants fumigated with HF, exposed to atmospheric fluorides, or treated with aqueous solutions of fluoride. Several metabolic effects are associated with leaf necrosis. The rate of oxygen uptake was increased in beans and gladiolus exposed to hydrogen fluoride. The effect increased with the duration of exposure and was most evident in young or expanding leaves (ref.107). The increased oxygen uptake in necrotic leaves of Polygonum orientale and Chenopodium murale was associated with an increased but altered glucose catabolism. The greater metabolic activity of necrotic tissues of soy beans induced by hydrogen fluoride was associated with decreased levels of sucrose, increased levels of amino acids and organic acids, increased CO_2 fixation, and an increased activity of phospho-enolpyruvate carboxylase in leaf homogenates (refs.108,109). The evidence indicated that enolase activity was inhibited in vivo, and the authors suggested that in the altered metabolic pattern, the utilization of ATP was increased and the observed increases in CO_2 uptake resulted from increased availability of ADP. Necrotic tissue itself, whether due to fluoride toxicity or mechanical or thermal injury, will produce increases in the O_2 uptake of adjacent tissue (ref.110). Therefore, it is difficult in many of these studies to ascertain which changes in metabolism are the causes or consequences of necrosis. The biochemical effects induced by fluoride that are not associated with chlorosis or necrosis are diverse and suggest that many different areas of metabolism can be affected. However, many of these effects have certain features in common. There is a threshold value of HF for each species, depending upon duration of exposure and HF concentration below which fluoride does not appear to affect the metabolism of the plant (refs. 111, 112). Above this threshold the metabolic response has both a magnitude and a duration, and the magnitude of the response may decrease or become reversed with the cessation of fumigation (refs. 113, 114, 115) or with time under long-term fumigations (refs. 116, 117). The translocations (ref.118) or inactivation of fluoride and the non-rate-limiting character of a fluoride-sensitive step or pathway could explain the existence of a threshold. The apparent recovery could also be explained by the removal of fluoride from a sensitive site.

Fluoride Metabolism

The metabolic fate of fluoride has been explored to some extent. Studies of pyruvic kinase activity *in vitro* have revealed that it can act as a "fluorokinase" and, consequently, can form fluorophosphate from fluoride with the utilization of ATP (ref.119). Monofluoroacetic acid has been isolated from several different plant species (refs. 120, 121, 122, 123); and in some species, ω-fluoro-oleic and other fluoro-fatty acids have also been found (ref.124).

The fact that some plants (Chailletacine) in Africa, especially *Dichapetalum cymosum* (Gifblaar) and other species, synthesize fluoroacetate ($F\ CH_2\ COOH$) raises the general question of whether the synthesis of the fluorine to carbon bond is a general property of plants. More recently another plant has been added to the list of plants capable of synthesis of fluoroacetate, *Acacia georginae* from Central Australia (refs. 125, 126).

Grass seedlings (Carter's Mixed Seed Invicta with rye grass) exposed to inorganic fluoride solutions do not take up appreciable amounts of fluoride until the concentration of fluoride is more than 1.0 mM (19 ppm) (ref.127). No formation of organic fluoride has been found even with exposure to 15.75 mM fluoride, indicating that there is no formation of fluoroacetate or similar compounds. Analyses were made of inorganic fluoride, fluoride acid labile on diffusion, total fluoride by combustion at 600°C, and alkali labile fluoride (ref.127).

Only one case has been reported in the literature in which actual uptake of fluoride has been estimated. Applegate, Adams and Carriker (ref.128) using seedlings of *Phaseolus vulgaris* (minus cotyledons) have determined the fluoride taken up at three stages of growth in different strengths of fluoride. There was little difference in uptake by the plants between light and dark growing conditions. When exposed to 1.0 mM F^-, the uptake of inorganic F was 22 μg/g. An increase to 10.0 mM gave an increase of ten times; and with 100 mM, the uptake was 2,846 μg/g. A rather steep rise in fluoride uptake for a long exposure takes place between 10.5 and 15.75 mM (ref.128).

Concentrations of total fluoride in the soil may reach 10.0 mM but only a small amount of this is present in a soluble form (ref.129). If all were present as calcium fluoride, the soluble amount would be 7.8 μg/g (7.8 ppm or 0.41 mM), well below the amount which gave a significant uptake.

The metabolism of fluoride in seedlings and small plants of *Acacia georginae* has been studied to determine the conditions under which the plant synthesizes fluoroacetate (ref.129). These studies showed that the uptake of fluoride from solutions of 0.525 to 1.05 mM (10 to 20 ppm) was not large. In 1 to 4 days it reached 8 ppm in the aerial parts and

16 ppm in the roots. Total fluoride was greater than inorganic fluoride. More "organic" fluoride was present generally in the roots than in the aerial parts. With higher concentration of fluoride, 10.5 to 15.75 mM (200 to 300 ppm), much larger amounts of fluoride were taken up, especially by the roots, and much more apparent organic fluoride was formed. pH influenced fluoride uptake with the pH effect being lowest at an initial pH 8.4 and highest at pH 4.0. Succinic dehydrogenase was not inhibited, and it was speculated that growth inhibition may be due to inhibition of phosphoglucomutase (ref.129).

Forage plants (alfalfa and orchard grass) and leafy vegetables (chard, spinach and romaine lettuce) have been fumigated with concentrations of about 0.60 to 0.83 $\mu g/m^3$ of hydrogen fluoride during their entire growth period (ref.130). These concentrations produced no fluoride-type markings on the leaves, although a relatively high concentration of fluorides accumulated in them (30 to 100 ppm). Microgram quantities of organic fluoride compounds were found in the plant materials but at a level too low for isolation and identification.

Fluorine Toxicity

Fluorine has a sharp, penetrating and characteristic odor, detectable in concentrations as low as 0.02 ppm. Because of this, inhalation of seriously toxic quantities is unlikely, unless the victim is trapped and escape from the fluorine is impossible. Fluorine gas is both highly irritating and toxic (ref.131). In concentrations ranging from 15,750 to 160 mg/m^3 (10,000-100 ppm), exposures of 5 minutes to 7 hours, to fluorine produced 54 to 100% mortality in rats (ref.132). All F_2 exposures at concentrations down to 315 mg/m^3 (200 ppm) for 3 hours produced 100% mortality within 14 days after exposure in all species. All the rats, mice, and rabbits were killed, and the guinea pigs showed a 90% mortality. No deaths occurred in the guinea pigs exposed 7 hours to 160 mg/m^3 (100 ppm), although 96% of the mice died. Upon prolonged exposure (170 hours) at 25 mg/m^3, the guinea pig and rat appeared to be equal in resistance to fluorine (50% mortality in each species).

The extraordinary reactivity of F_2 may explain the toxicological difference in the effects of F_2 and HF when each is mixed in low concentration in moist air. Fluorine may react with water vapor to form OF_2 which may have primary responsibility for the severe respiratory irritation when F_2 is in air. The TLV's reflect the intrinsic relative toxicities; the current threshold limits are 0.1 ppm for F_2 and 3 ppm for HF. More recent work (Dr. M. Keplinger, private communication)* indicates an LC_{50} for fluorine with rats of 700, 390, 270 and 185 ppm for 5, 15, 30 and 60 minute exposures.

*Dr. M. Keplinger, University of Miami, Miami, Florida.

SECTION IV

A SUMMARY OF CURRENT RESEARCH ON ANIMAL INTOXICATION BY INORGANIC FLUORIDES

Oregon State University is currently performing research on the pharmacology and metabolism of inorganic fluorides. The research utilizes the exposure techniques described in Section VI of this report. The following is a brief summary of this effort.

Toxicity of Nitrogen Trifluoride

Intraperitoneal injection of undiluted gaseous NF_3 into rats at a level of 9.0 to 9.3 ml/kg body weight resulted in 100% mortality. In contrast, injection of 7.2 ml/kg of NF_3 resulted in 100% survival. Thus the LD_{50} for NF_3 by intraperitoneal injection appears to be 0.35 to 0.4 mmole/kg body weight. Exposure of rats to NF_3-air mixtures by inhalation gave an LC_{50} of 10,000 ppm of NF_3 for a 60-minute exposure period.

The exposure of animals to a lethal amount of NF_3 causes massive formation of methemoglobin. Death could be correlated with methemoglobin levels which were in excess of 60% of the total hemoglobin present. If an animal survived at the peak level of methemoglobin, permanent survival was generally assured. Methemoglobin reductase, which is pyridine nucleotide dependent, is known to cause a rapid reduction of methemoglobin to hemoglobin. We found that within 60 to 90 minutes after exposure of rats to NF_3, a majority of the methemoglobin had been reduced to hemoglobin. *In vitro* experiments with whole blood showed a dependency upon glucose catabolism for the reductase activity.

Thus, NF_3 appears unique in being both a very stable compound and a strong oxidizing agent which is very susceptible to rapid interaction with hemoglobin. Survival of NF_3-exposed animals, then, appears very dependent upon methemoglobin reductase which requires oxygen and glucose for its reduction capacity.

Toxicity of Nitrogen Tetrafluoride

In this laboratory, LC_{50} values established for N_2F_4 with Sprague-Dawley male rats are comparable with those described by Stevenson (private communication, 1965)[1]. In our experiments with

[1] Dr. Kenyon Stevenson, Head, Chemical Engineering Section, Redstone Arsenal Research Division, Rohn & Haas Co. Huntsville, Alabama.

exposure times of 30 and 60 minutes, the LC_{50} values were 1,200 and 400 ppm, respectively. The exposures were made in a 3-liter glass chamber while employing a very rapid turnover of the N_2F_4-air chamber atmosphere. The chamber atmosphere was monitored continuously with a 10-cm infrared cell and a Beckman IR-5A spectrophotometer. In preliminary experiments, considerable difficulty was experienced in attempting to generate an N_2F_4 atmosphere in a metal 100-liter exposure chamber when the chamber atmosphere flow rate was 10 liters per minute. Using the glass 3-liter chamber, many of the parameters necessary for a stable N_2F_4 -air atmosphere were established and routinely a 35-minute exposure to 10,000 ppm of N_2F_4 in air is a lethal exposure for adult rats. Animals exposed under these conditions will either die in the chamber or within a very short period following their removal from the chamber. Severe gasping response is noted, and its cause is still undetermined. While a substantial amount of edema and irritation can be observed in the lungs of exposed animals, there seems to be little evidence of gross irritation in their upper respiratory tract.

Tetrafluorohydrazine Degradation in Air

The effect of oxygen and water vapor on the stability of N_2F_4 in air is being studied. Preliminary experiments indicated that results of the kinetic study of the decomposition of N_2F_4 in air were very erratic and nonreproducible when carried out in infrared cells fabricated of glass or metal and equipped with sodium chloride windows. Therefore the cell used was fabricated of Teflon and equipped with silver chloride windows. The relative concentrations of N_2F_4, NOF and NO_2 were determined by their infrared absorption at wavelengths of 10.45, 5.4 and 6.15 mμ, respectively. When glass infrared cells were used, in the earlier experiments, an absorption maximum at 9.75 mμ was observed. The complete spectrum was identical to that for SiF_4. In virtually all of the experiments carried out in the infrared cell constructed of Teflon, NOF was formed as N_2F_4 disappeared. Subsequently, NOF disappeared coincidentally with the formation of NO_2. NO_2 formation usually did not begin until the N_2F_4 concentration approached a minimum value. NO_2 then appeared to persist, and its concentration reached a steady level after the complete disappearance of N_2F_4 and NOF. In general, these experiments suggest that decompositon of N_2F_4 will proceed at a rate which is at least, in part, dependent upon the amount of oxygen available to the reaction. In a system made relatively, although not necessarily completely, free of oxygen, the degradation of 1% N_2F_4 in helium or nitrogen requires approximately 90 minutes. If 1% N_2F_4 is mixed with air, the complete disappearance of N_2F_4 requires only 20 minutes. While this investigation is continuing, evidence to date indicates that the exposure of biological systems to N_2F_4 may result in the exposure of not only N_2F_4 but also NOF and NO_2, depending on the actual exposure conditions. Other workers have described the toxicity of NO_2 (ref.133), but virtually nothing is known concerning the toxicity of NOF.

Toxicity of Chlorine Trifluoride

Rats were exposed to ClF_3 under dynamic exposure conditions, with sufficient flow rates of ClF_3 -air mixture to ensure that an intact atmosphere of ClF_3 flowed through the exposure chamber. The ClF_3 -air atmosphere can, therefore, be maintained without decomposition products that were detectable by an infrared spectrophotometer and a 10-cm flow cell. With the dynamic flow exposures, 400 ppm of ClF_3 in air will cause 100% mortality to rats within a 30-minute exposure. The concentration of ClF_3 can be raised to 800 to 900 ppm without appreciably decreasing the time of exposure to cause death. The duration of exposure required for lethality, then, may be of greater importance than the concentration of ClF_3 in the 400 to 900 ppm range. The degree of damage necessary for lethality may occur in less than 30 minutes, yet this length of time may be necessary for death to occur. Attempts to expose animals at lower concentrations for a longer period of time were complicated by a decrease in ClF_3 concentration and an increase in degradation products being formed during the exposures. Exposed animals suffer great distress and injury; their hair becomes very brittle and often turns yellowish; all mucous membranes are extremely inflamed, and corneal opacity often develops in animals which survive the exposure. Animals exposed to ClF_3 do not form measurable levels of methemoglobin.

SECTION V

EXPOSURE TECHNIQUES

The exposure of biological systems to gaseous inorganic fluorides presents difficult technical problems. A major effort has been expended to solve many of these problems. A detailed report of a study in this laboratory on equipment, material compatability and procedures has been described (ref. 134). The exposure conditions described in this report are a result of our previous study.

General Safety Considerations

The toxic and reactive character of the inorganic fluoride gases required that protection of personnel be carefully planned. To assure the effectiveness of the methods of protection, a specific system of discipline is adhered to at all times, regardless of the degree of hazard.

Ventilation

All work with the inorganic fluorides, including plant exposures, is being carried out in a 4.6 square meter (50 square foot) walk-in hood, vented at a rate of 85 cubic meters (3,000 cubic feet) per minute, independent of all other hood systems in the building. Two bench hoods of very high capacity are used for support operations and storage of gas cylinders.

Tank Barricade

Tanks or lecture bottles of the inorganic fluoride gases in actual use are housed in a barricade fabricated of 6.4 mm (1/4 inch) plate steel. The barricade serves as a heat sink and shield and can be expected to contain a substantial fire long enough for evacuation of the laboratory. Tank and delivery gages are visible through a safety-glass port, and valve controls are extended several inches beyond the barricade wall. A shielded bulb lights the interior. A removable circulating water bath with an external heat-exchanging system controls the vapor pressure of ClF_3 and BrF_5. Gases from leaking tank valves or other fittings are exhausted from the barricade by continuous negative pressure ventilation provided by a Gast pump, Model No. 0211, lubricated with Kel-F oil. Access to the box is by removal of its front panel which is held in place with wing nuts.

Disposal of Gaseous Wastes Containing Fluorine

All fluorine-containing effluents, regardless of dilution, are combusted by a Terrill burner mounted in a 3-inch steel pipe. Incoming lines discharge into the pipe just below the flame, and the combustion products are vented directly into the exhaust of the walk-in hood.

Direct Personnel Protection

When working with cylinders containing the inorganic fluorides and mechanical parts which may be filled with toxic materials, personnel are required to wear shoulder-length neoprene gloves and an airline mask. The masks are supplied with filtered air under positive pressure from an external source. Personnel are not allowed to work alone when handling inorganic fluoride gases.

Fire Protection

A 1-inch fire nozzle adjusted to a fine spray is permanently mounted in the walk-in hood to deluge the barricade and distribution area should a fire start. A high volume of water as a fine spray will react smoothly with these agents to form more easily disposable products, and will carry them away from burning areas. A second nozzle with hose is situated in the laboratory where it can be used as a personnel decontamination shower or be detached quickly for firefighting at any point in the laboratory.

Methods and Materials

Sources of Inorganic Fluoride Gases

NF_3, N_2F_4, ClF_3 and BrF_5 were provided by Air Products and Chemicals, Allentown, Pennsylvania.

Exposure Chambers

The main exposure chamber and its airlock are constructed of aluminum. Because of the great surface area involved, it is often desirable to install a Teflon lining during the use of certain of the more active agents such as N_2F_4, ClF_3 and BrF_5. A 1.6 mm (1/16 inch) thick observation window of a transparent formulation of Kel-F was employed. (Allied Engineering and Production Corporation of Alameda, California). A second exposure chamber with a 3.6 liter volume was constructed of brass, with a Kel-F window, and Teflon end plates. Some experiments in this chamber have been made with a Teflon liner of 0.25 mm (0.010 inch) film,

for exposures to interhalogens.

The main exposure chamber measures 45.7 cm by 45.7 cm by 73.7 cm (18 by 18 by 29 inches) and is constructed of aluminum (Figure 1). A cast aluminum door sealed with Teflon gasketing is bolted into flanges at one end; it is rarely removed. A Kel-F window provides a view of specimens during exposure. After dilution to a desirable concentration, the toxic gases are distributed throughout the chamber by a perforated pipe extending across the top upper front of the chamber and removed by a similar pipe across the lower rear of the chamber.

The chamber is equipped with an airlock which enables rapid introduction and removal of experimental materials. In tests with CO_2 and NF_3 atmospheres, operation of the airlock caused no change in chamber concentration as measured by infrared spectrophotometry.

Plant seedlings are placed in a five-compartment cage which is carried on tracks through the airlock and into the exposure chamber. A polished rod directed by a machined nylon bushing in the center of the outer airlock door is used to push the carriage into the exposure chamber. The rod is disengaged from a fitting on the carriage by rotating and pulled back out of the path of the inner airlock door, which is then lowered and locked. The process is reversed for removal. Any chamber atmosphere carried into the airlock by the cage is purged by compressed air.

The contact surface between the airlock door and the body of the cage is metal against metal and has been hand-lapped to insure against leakage. In this way, the reactivity of organic materials is avoided, and the cold flow characteristics of a Teflon gasket under pressure are also avoided. Four sampling probes are located at various points in the chamber for detection of atmosphere distribution.

The auxiliary 3.6-liter chamber is fabricated from a 20.3 cm (8 inch) section of 15.2 cm-(6 inch) brass pipe, and has Teflon lining and Teflon end plates and a Kel-F window. It is used for pilot experiments, high-concentration exposures of seeds or microorganisms, and evaluation of materials.

Atmospheres from either exposure chamber may be passed continuously through a flow cell of an infrared spectrophotometer by the slight back pressure of the chamber.

Delivery of Inorganic Fluoride Gas to the Dilution and Distribution System

Control of flow rates less than 50 ml/min is achieved with delivery pressures below 5 psi. Tank pressures of NF_3, N_2F_4, and OF_2, which

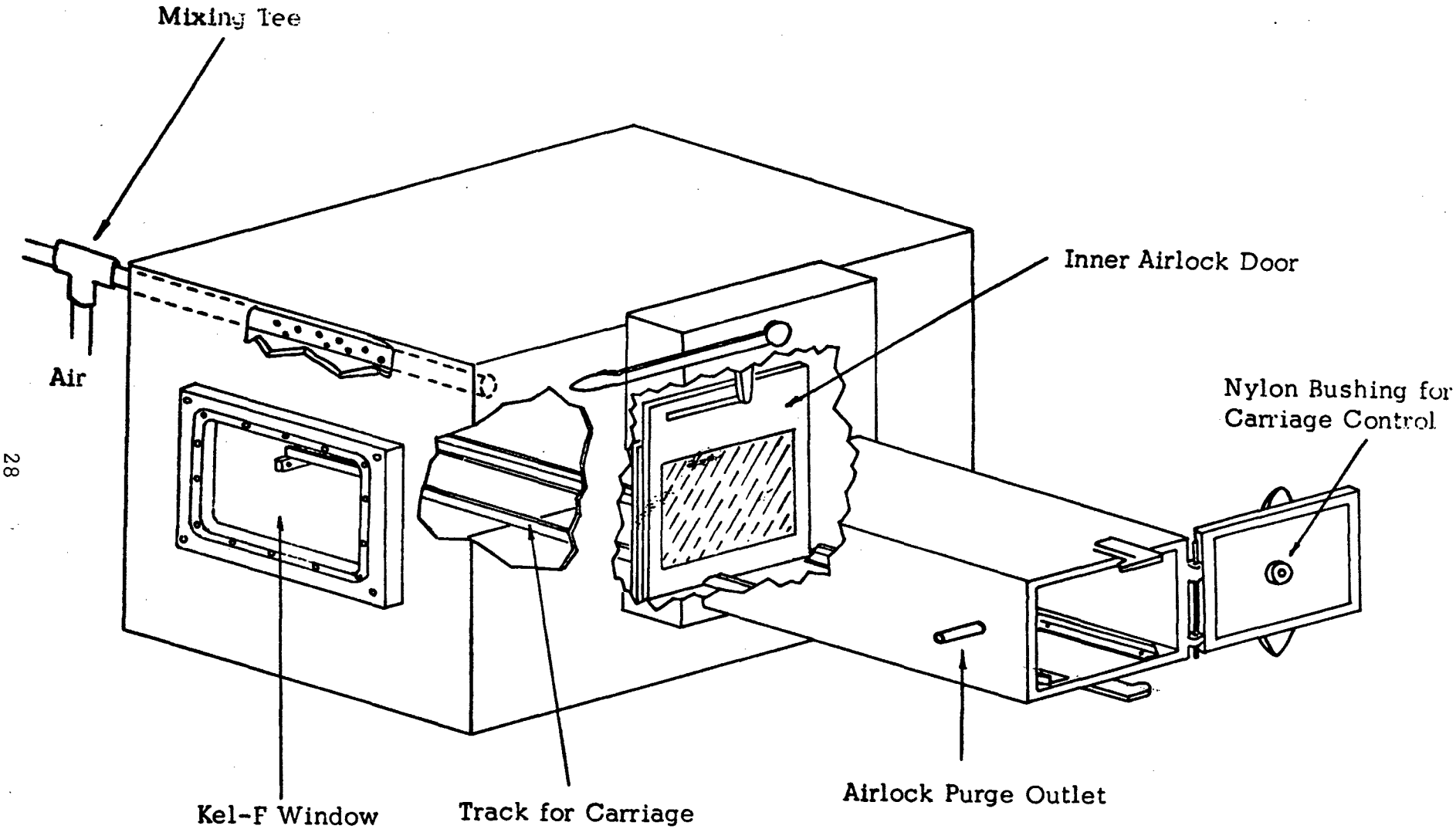


Figure 1. Exposure chamber and airlock.

may reach several hundred pounds, are stepped down with three to five Whitey No. IRS4 semiregulating valves in series. Unless downstream obstruction occurs, this method gives a uniform flow rate not possible with diaphragm regulators. The pressure range desired can be met by adjustment of successive valves in the cascade to nearly closed positions. Occasionally, it is necessary to vent an appropriate fraction of gas at the third stage to achieve control. This vent also protects against pressure excesses during the period of adjustment. Reduction of pressure across a given valve is from twofold to fivefold in barely opened valves. If downstream resistance is not changed, very little adjustment is required for a balanced system.

The delivering pressure of ClF_3 and BrF_5, which are stored as liquids, is adjusted by controlling temperature and therefore vapor pressure, of the materials.

Control of Gas Flow Rates

Metering of undiluted gases into the dilution system is controlled either by a series of parallel orifices used singly or in combination or by the regulating valve (Whitey No. 21RS4) which bypasses the orifices. Each orifice or valve is preceded by a filter of sintered stainless-steel 7 micron in pore size. (Nuclear Products Company, Cleveland, Ohio). The filter prevents clogging by metal fluoride particles shed from the lining of the tubing. When backing pressures are maintained constant, variation in flow rate through a given orifice or valve gap is minimal. The orifices are designed to minimize erosion effects by impinging gases upon the orifice diameter.

Flow of nitrogen gas for dilution is controlled by a Nupro fine-metering valve in a range usually between 0 and 1500 ml/min. Division of the fluoride-nitrogen stream is done with two Whitey No. 3RS4 metering valves, one directed into the exhaust burner, the other to the final mixing point. Flow rate of the dry air for the final dilution is controlled by a Nupro fine-metering valve, at rates up to 10 liters/min. Since the methods of the metering of undiluted gas usually only approximate a desired rate, the final adjustment of concentration may be made by changing any of the other three flow controls.

Measurement of Gas Flow

A mass flow meter (LF-20, Matheson Company, East Rutherford, New Jersey), obtained for continuous on-stream measurement of flow, is used to measure undiluted gases at low flow rates. It measures flow by measuring the amount of heat transport from a heat source by gas molecules passing through a tube and can be calibrated directly or mathematically as a function of gas heat capacity.

Mixing of Air with Gaseous Inorganic Fluorides

The inorganic fluoride-nitrogen stream is mixed with air in a final mixing chamber which is an integral part of the exposure chamber. The nitrogen-inorganic fluoride mixture is discharged through a 6.4-mm (1/4-inch) line into a concentric 19.2-mm (3/4-inch) line carrying diluent air at 5 to 20 l/min. The mixture is then moved directly into the exposure chamber.

Infrared-Cell Materials

Continuous monitoring of chamber atmospheres, usually by infrared spectrophotometry, is necessary for good control of air-inorganic fluoride exposures. Brass or Monel cell bodies apparently do not contribute reaction products to the gas under analysis, although preliminary evidence suggests that ClF_3 decomposes more rapidly in presence of brass. Silver chloride windows (Harshaw Chemical Company, Cleveland, Ohio) are sufficiently nonreactive that they will tolerate the gases used in this research.

Standard Curve of Infrared Optical Densities of NF_3 Dilutions and its Use

A standard curve for the infrared absorption of NF_3 at a wavelength of 11 μ has been established with a known dilution of 1430 parts NF_3 per million by volume. The NF_3-helium standard was supplied by Air Products and Chemicals, Allentown, Pennsylvania. The optical densities of the standard gas at light paths of 1, 5 and 10 cm fall on a slope which represents concentrations up to 14,300 ppm in a 1-cm cell. Similarly, the concentration of 1430 ppm in a 1-cm cell has an optical density identical to 143 ppm in a 10 cm cell. The calibration curve is shown in Figure 2. The precision of the flow-dilution method for achieving desired gas dilutions is illustrated by a series of infrared measurements of experimental atmospheres whose concentrations were previously estimated on the basis of relative flow rates. These are plotted as estimated concentration versus optical density in relation to the standard curve in Figure 2. A Beckman IR-5A infrared spectrophotometer coupled with a strip chart recorder is used for these measurements. Monitoring of concentrations of other agents depends on standards prepared in this laboratory and chemically confirmed.

Inorganic Fluoride-Air Atmospheres

Exposures of plant seedlings to inorganic fluoride-air atmospheres were carried out in the exposure chamber described in Figure 1. The experimental atmospheres were established as described above. Infrared spectrophotometry was used to monitor both the concentration of the inorganic fluoride and its purity after mixing with air.

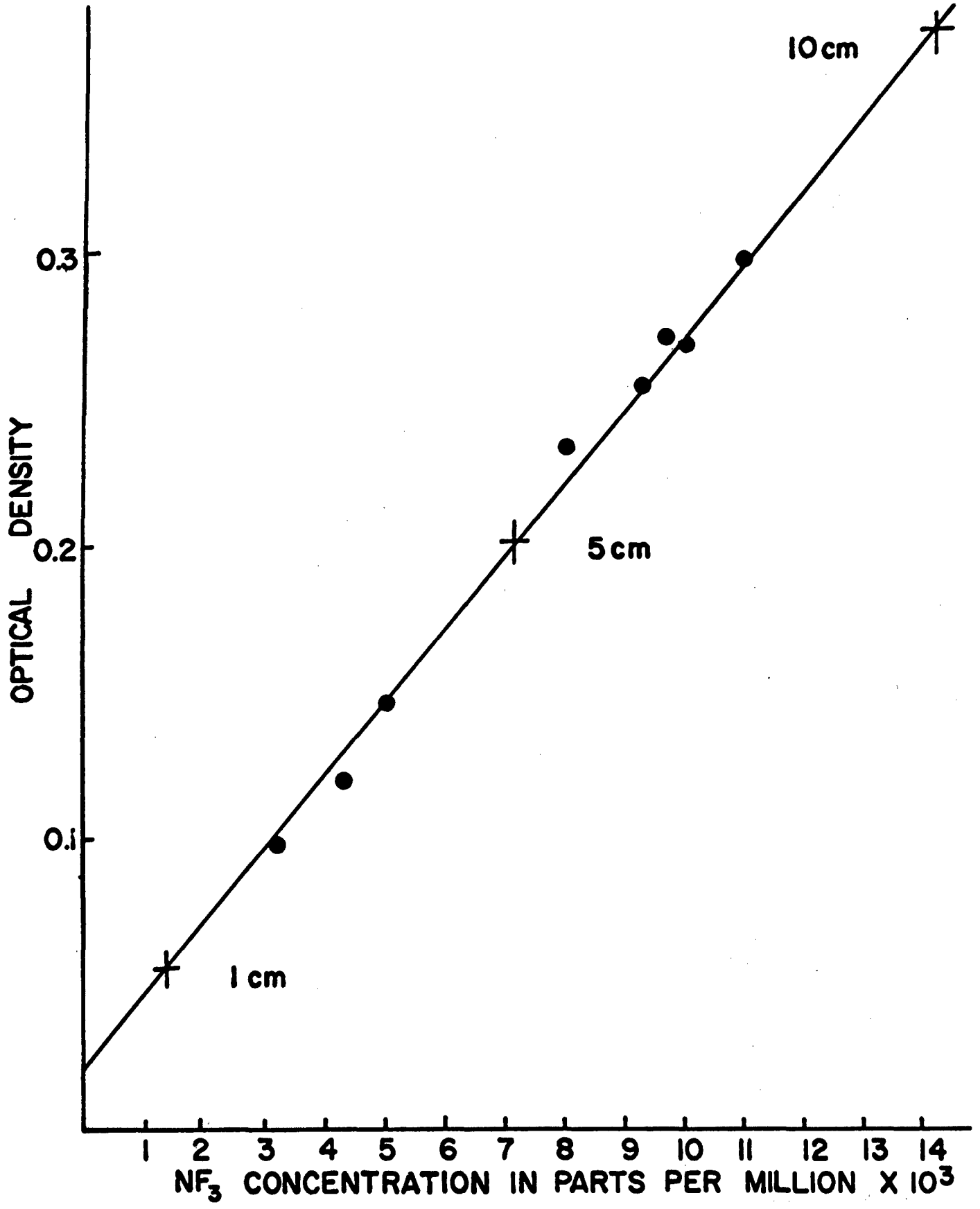


Figure 2. Standard curve for infrared absorption of NF_3 . Wavelength, 11μ

Infrared Spectrophotometry of Inorganic Fluoride-Air Mixtures

The following data illustrate the relationships between the concentration of inorganic fluoride as measured by a mass flowmeter and optical density of the particular inorganic fluoride-air mixture formed in the exposure chamber. A 10-cm flow cell equipped with silver chloride windows was used with a Beckman IR-5A infrared spectrophotometer.

TABLE II

Concentration of Inorganic Fluorides in Air Versus Infrared Optical Density Measurements

	Inorganic Fluoride								
	NF_3			N_2F_4			ClF_3		
Wavelength(μ)	11.0			10.45			14.1		
ml of inorganic fluoride per liter of air	1.4	7.2	14.3	1.8	7.3	14.6	1.1	4.5	6.8
Optical density reading	0.05	0.20	0.38	0.15	0.36	0.65	0.30	0.80	1.15

Procedure Used in Exposing Seeds to Inorganic Fluoride Oxidizer-Air Atmospheres

Exposure of seeds to atmospheres of inorganic fluorides was carried out in the 3-liter Teflon-lined exposure chamber. Dry seeds were exposed on metal mesh screens in such a manner that the atmosphere circulated into the chamber and then through the metal mesh screen supporting the seeds.

For these experiments, 800 seeds were exposed per experiment. Four-hundred of the exposed seeds were immediately placed on germination towels after exposure and another 400 were washed five times in fluoride-free distilled water within 10 minutes after being removed from the exposure chamber. When washed, these seeds were placed on germination towels.

Types of Seeds and Germination Procedures for Germination

The five types of seeds used for germination tests were bean (Phaseolus vulgaris), corn (Zea mays), squash (Cucurbita sp.), sudan grass (Sorghum vulgare sudanense), and pea (Pisum sativum). The seeds were germinated on

germination towels 9 1/2 by 16 1/2 inches. The towels were soaked in the different concentrations of fluoride-containing solutions or in distilled water for 2 hours, then wrung to very damp for bean, corn, and pea, and slightly damp for squash and sudan grass. Fifty seeds were placed on each towel (100 for sudan grass). A total of 400 seeds per concentration of solution was used, with a control of 100 grown on towels soaked in distilled water. Two towels were smoothed out, the seeds placed on them, one towel placed on top and folded along the bottom, and the three towels rolled and placed in an all-plastic container.

The seeds were germinated at alternating temperatures of 20°C for 16 hours, and 30°C for 8 hours, with the exception of pea which was germinated at a constant 20°C in germination chambers. The germination periods were bean - 7 days, corn - 7 days, squash - 7 days, sudan grass - 10 days, and pea - 8 days.

Criterion for Germination

The seedlings were counted according to the seedling interpretation listed in Testing Agricultural and Vegetable Seeds (ref. 135). The normal and abnormal characteristics listed are given in the Appendix.

Inorganic Fluoride-Water Reaction Mixtures

Gaseous N_2F_4, ClF_3, and BrF_5 were mixed with distilled water to give reaction mixtures which were examined for their toxicity. The reaction was carried out in a sealed, inverted 3.79 liter polyethylene bottle. Gaseous inorganic fluoride was delivered into the bottle near the inverted bottom of the bottle by Teflon tubing which passed through the wall of the bottle. The tubing made a gas-tight seal in the wall opening of the bottle. In this manner, all of the inorganic fluoride-water reaction products were retained in the sealed bottle. Fluoride analysis was carried out to determine the concentration of fluoride. The reaction mixture was adjusted to the desired concentration of fluoride ion in ppm.

Irrigation of Plants with Inorganic Fluoride-Water Reaction Mixtures

Seeds of bean, corn, pea or squash were planted in plastic pots containing vermiculite. Four pots, each containing four plants (except sudan grass pots which contained 20 plants each) were used for each experiment. One control pot and 2 experimental pots for a total of 12 plants were utilized. During a growth period of 10 days, each pot was watered with a nutrient (inorganic salts) solution (ref. 136). After the

10-day growth period each of the sets of plants to be treated was watered with 100 ml of inorganic fluoride-water reaction mixture each day for 2 days. None of the solution came into direct contact with the leaves. The controls were watered with 100 ml of tap water each day for 2 days. Both treated and control plants were subsequently irrigated with the nutrient solution of inorganic salts every day.

Growth Conditions of Seedlings Exposed to Inorganic Fluoride-Air Atmospheres

Seed were planted in vermiculite which was contained in rectangular aluminum containers. During germination, the vermiculite was kept moistened with an inorganic salt solution. The seeds, after being planted in vermiculite, were germinated for 9 to 11 days in a plant-growth chamber maintained at 30°C for 8 hours in the light and at 20°C for 16 hours in darkness. After the 10-day growth period, the plants were measured for height and then used either as control plants or exposed to an inorganic fluoride-air atmosphere.

Physical condition of the plants was judged and growth measured and recorded 12 to 18 days following treatment with the inorganic fluoride-water solutions. Fluoride analysis of the plant roots, stems and leaves was by the method described in the following section.

SECTION VI

FLUORIDE ANALYSIS

Weinstein et al. (ref.137) described a rapid and semi-automated procedure for the analysis of fluoride in air and plant tissue samples. This procedure is based upon the use of a Technicon AutoAnalyzer and a lanthanum-alizarin complexone color reaction. The method is comparatively rapid since analytical data on ashed samples can be obtained in approximately 13 minutes. In our laboratory a modification of this procedure was employed and was found to give very reproducible results with ashed samples containing a total of 2.5 to 100 μg of fluoride ion per sample. A more detailed description of this procedure and other fluoride analysis procedures developed in this laboratory can be found in another report (ref.138).

Preparation of Samples for Ashing

For fluoride analysis, tissues of animals or plants were dried at approximately 60°C by infrared lamps overnight. After drying the

samples were ground in a Wiley mill (Arthur Thomas Company, Philadelphia, Pennsylvania). One gram of each dried and ground sample was placed in nickel crucibles with 1.5 ml of 1 M lithium hydroxide and 1 ml of 0.2M magnesium succinate. The samples were again dried and the crucibles covered and fired overnight at 400°C in a muffle furnace equipped with an electronic temperature controller (Thermolyne Corporation, Dubuque, Iowa).

Reagents

1. Alizarin-complexone, 0.01 M was prepared by suspending 962.5 mg of alizarin* in 100 ml of water and 2 ml of ammonium hydroxide (28% NH_3) added to dissolve the alizarin. The pH of the solution was then lowered with 2 ml concentrated acetic acid and the solution volume then brought to 250 ml with water. The pH of the final solution was 5.3.

2. Lanthanum nitrate, 0.01 M.

3. Sodium acetate buffer, pH 4, prepared by adding 100 ml of concentrated acetic acid to 60 grams of sodium acetate $.3H_2O$ in 500 ml of water. The total volume was then adjusted to 1.0 L.

4. The lanthanum-alizarin complexone reagent was comprised of 200 ml of water, 200 ml of acetone, 50 ml of buffer, 18 ml of alizarin reagent, and 18 ml of the lanthanum nitrate solution.

Procedure for the Auto Analyzer Method

Ashed samples for fluoride analysis are placed in 18 x 150 mm test tubes and dissolved in 1 ml of 2 N $HClO_4$ and then diluted with deionized distilled water to a 10 or 12 ml volume. The samples are placed in every sixth position of the large sampler shown in Figure 3. Intervening positions of the sampler rack are occupied by test tubes containing distilled water. Each sample is pumped by a proportional pump into the heated and revolving helix of the Digestor unit for 1 minute at a rate of 4.7 ml/min. Air is pumped with the sample at a rate of 0.82 ml/min. Sulfuric acid (60%)

*Obtained from Hopkin and Williams Ltd. Freshwater Road, Chadwell Heath, Essex, England.

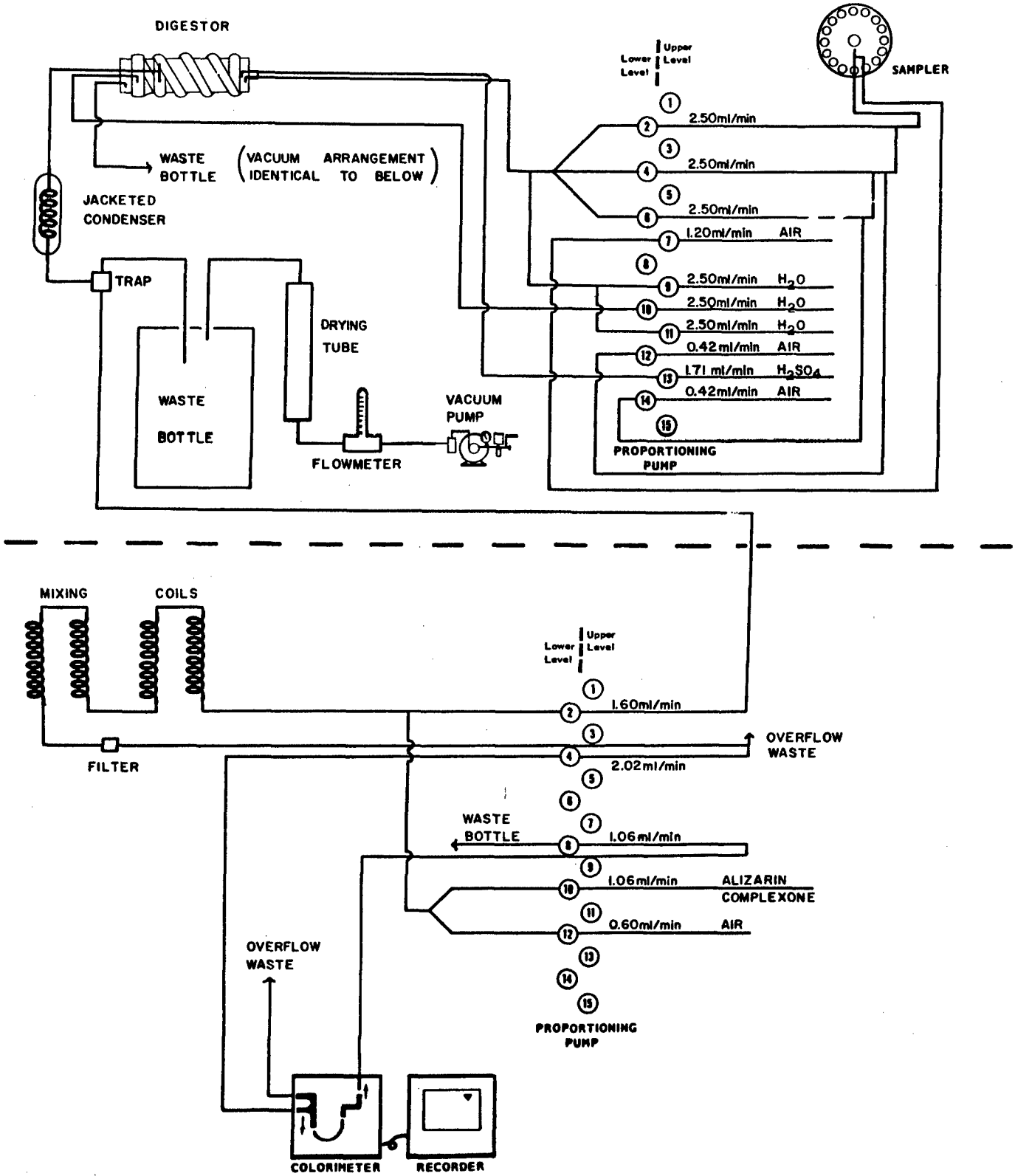


Figure 3. Flow diagram for semiautomated fluoride analysis by the Weinstein method.

at a rate of 1.7 ml/min is pumped concurrently into the revolving helix. The internal temperature of the helix is maintained at 132°C as measured with a thermometer in the center of the helix. Hydrogen fluoride and water vapor are evolved as the sample-sulfuric acid mixture passes through the helix. The evolved HF and water vapors are condensed in a water-cooled condenser and a portion of the condensate (1.0 ml/min) mixed with a stream of the lanthanum-alizarin complexone reagent (1.06 ml/min). Color development occurs as the stream passes through the time-delay coils and is then pumped through the flow colorimeter and the optical density of each sample is measured at a wavelength of 625mμ. Samples containing known amounts of fluoride, 2.5 to 100 μg/20 ml sample volume, are used each day for standards. The time elapsed after the sample is introduced into the automatic system until a signal is registered on the recorder (indicating the amount of fluoride in the sample) is approximately 13 minutes.

In Figure 4 is shown the linear response in optical density units compared to the fluoride content of samples analyzed by this method.

The analysis of fluoride ion by the method described was satisfactory for the determination of fluoride in plant tissues. In most cases, one gram samples of dried plant material were sufficient for the determination of fluoride ion by this method. During the analysis of a set of samples the linear response of the instrument to fluoride ion was checked with samples containing known amounts of fluoride ion. In this manner a check was maintained on the reliability and reproducibility of the analytical procedures.

The rate of loss of fluoride by volatilization from samples dissolved in 0.1 N $HClO_4$ was measured. Ashed samples were dissolved in 1 ml of 2 N $HClO_4$, diluted to 20 ml and analyzed at varying time intervals for fluoride content with the Auto Analyzer. No loss of fluoride from samples was detected 60 minutes after being solubilized. After 90 minutes, it was possible to show a detectable loss of fluoride and after 47 hours, approximately 17% of the fluoride was evolved at room temperature. The insignificant loss of fluoride within the first two hours after perchloric acid treatment of the samples made it possible to prepare 20 to 30 samples for analysis at one time.

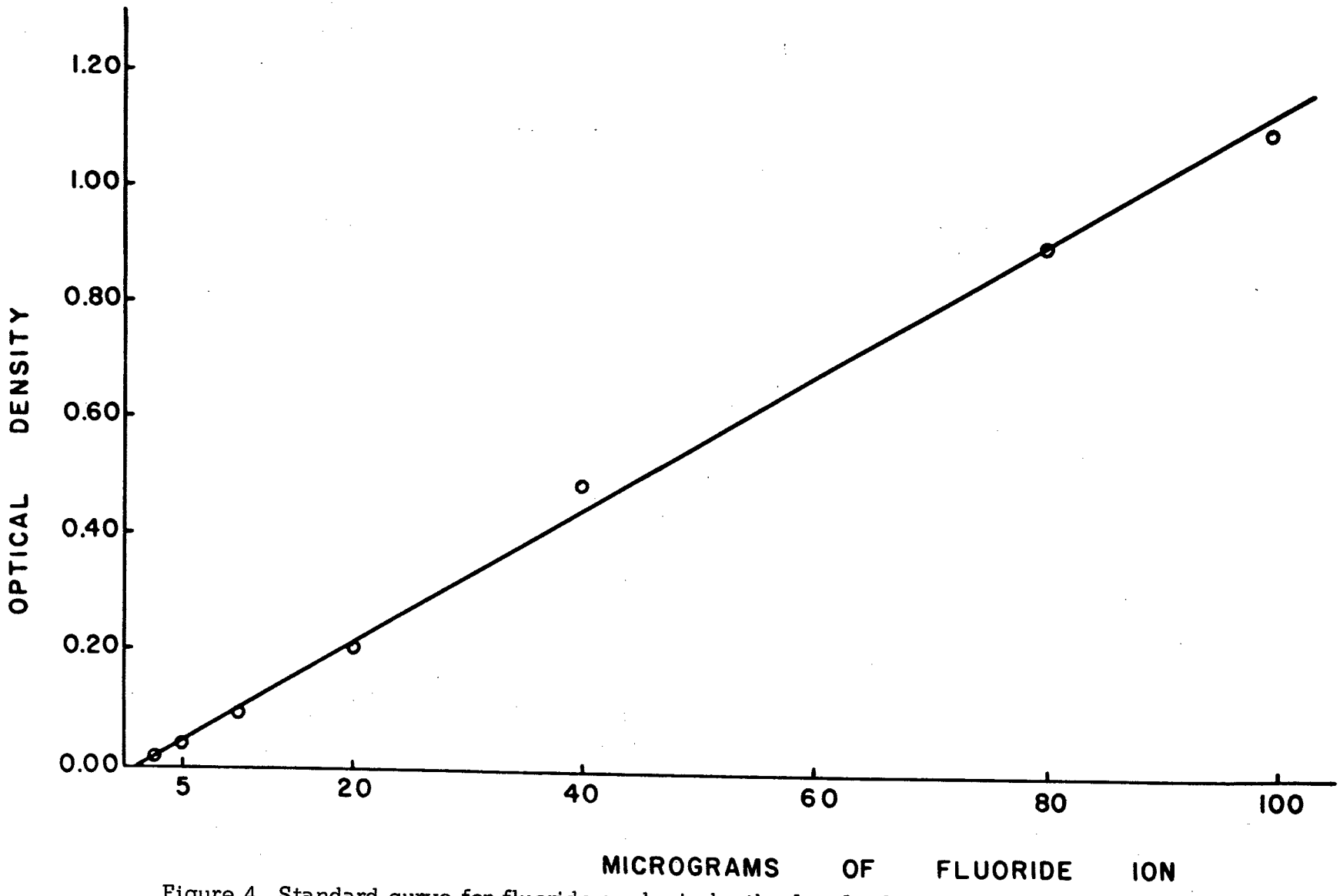


Figure 4. Standard curve for fluoride analysis by the AutoAnalyzer method. A plot of optical density versus micrograms of fluoride ion per 20 ml sample.

SECTION VII

RESULTS

Effects of Sodium Fluoride on Seed Germination

The sensitivity of seeds of various plant species to solutions of sodium fluoride during germination is shown in Table III. Bean and sudan grass seeds were very sensitive to the presence of sodium fluoride during germination. Approximately 50% of the bean seeds did not germinate in the presence of 0.25 gm/liter of sodium fluoride. A little more than 0.5 gm/liter of sodium fluoride was necessary to inhibit by 50% the germination of sudan grass seeds. Pea and squash seeds were intermediate in their sensitivity to inhibition by this fluoride. Corn seeds were found to be considerably more resistant to inhibition as 2.5 gm/liter of sodium fluoride was required before a 50% inhibition of germination could be achieved. The fluoride content of these seeds was measured at the end of each germination period (Table III). The level of fluoride found in those seedlings which grew when approximately 50% of the seeds germinated in the presence of sodium fluoride ranged from about 40 μg/gm dry weight in bean seeds to about 250 μg/gm dry weight in the germinated sudan grass seeds. We conclude that in part the sizes of the seeds and the nature of the seed coat may be factors in the level of fluoride found in seeds after germination in the presence of sodium fluoride solutions.

Effect of Hydrogen Fluoride on Seed Germination

Solutions of hydrogen fluoride were found to cause inhibition of seed germination at lower concentrations than solutions of sodium fluoride (Table IV). Fifty percent inhibition of seed germination by HF solutions was achieved with as low as 0.025 gm/liter of hydrogen fluoride in experiments with sudan grass seeds and with a concentration as high as 0.25 gm/liter with pea seeds. The pH of the hydrogen fluoride solution may affect the germination of the seeds. Sodium fluoride solutions at 1.50 or 2.50 gm/liter had a pH of 6.4. Solutions of hydrogen fluoride at 0.050 gm/liter had a pH of 3.1 and a concentration of 0.25 gm/liter resulted in a pH of 2.6. The fluoride content of seeds germinated in the presence of hydrogen fluoride was determined (Table IV). It was found that approximately the same amount of fluoride was present in those seedlings resulting from 50% seed germination, whether the germination was carried out in a solution of sodium fluoride or hydrogen fluoride. For example, it was found that a 50% inhibition of seed germination with hydrogen fluoride solutions resulted in a fluoride content ranging from 56 μg/gm dry weight to 412 μg/gm dry weight. As was noted previously, sodium fluoride solutions which also cause 50% inhibition of germination resulted

TABLE III

Effect of Sodium Fluoride Solutions on Seed Germination

Plant Species	Sodium Fluoride Concentration (g/liter)	Percent Germination	Seedling Fluoride Content μg/g Dry Wt.
Bean	0	87	8
	0.10	60	11
	0.25	42	43
	0.50	30	73
	1.00	23	--
	2.50	0	--
	5.00	0	--
Corn	0	99	2
	0.10	97	--
	1.00	87	--
	2.50	48	77
	5.00	0	--
	10.00	0	--
Squash	0	74	4
	1.00	62	157
	2.50	25	509
Sudan Grass	0	71	< 1
	0.25	61	60
	0.50	54	236
	1.00	23	>1,500
	2.50	0	
Pea	0	94	2
	0.50	69	135
	0.70	41	---
	1.00	18	192
	2.50	0	930

-- Samples not analyzed

TABLE IV

Effect of Hydrogen Fluoride Solutions on Seed Germination

Plant Species	Hydrogen Fluoride Contentration (g/liter)	Percent Germination	Seedling Fluoride Content µg/g Dry wt
Bean	0	87	8
	0.050	85	21
	0.100	86	--
	0.175	49	74
	0.240	31	89
	0.470	0	242
Corn	0	99	2
	0.025	92	--
	0.082	91	31
	0.097	69	34
	0.10	72	--
	0.15	55	--
	0.25	19	--
	1.00	0	--
	2.50	0	--
Squash	0	74	4
	0.050	74	54
	0.10	65	155
	0.25	57	412
	0.30	47	
Sudan Grass	0	71	1
	0.005	65	25
	0.010	62	40
	0.025	58	56
	0.038	0	--
	0.050	0	--
Pea	0	94	2
	0.050	94	20
	0.10	91	42
	0.25	43	199

-- Samples not analyzed

in a fluoride content ranging from 40 μg/gm dry weight to 250 μg/gm dry weight. While much higher concentrations of sodium fluoride than that of hydrogen fluoride were necessary to cause a 50% inhibition of germination of corn and squash seeds, comparable levels of fluoride were found in the seedlings after germination. These results would indicate that the actual pH of the germinating solution may not play a major role in inhibition of seed germination in the presence of hydrogen fluoride or sodium fluoride solutions.

Effect of Nitrogen Trifluoride Exposures on Seed Germination

Exposure of dry seeds to an atmosphere of nitrogen trifluoride (undiluted) was found to inhibit the germination of all seeds examined (Table V). However, the exposure of dry seeds to an undiluted NF_3 atmosphere requires an exposure time of about one hour or longer for an indication of inhibition. For example, it was necessary to expose bean seeds for 6 hours to an undiluted NF_3 atmosphere before 50% inhibition of germination could be obtained. All other seeds examined were considerably more sensitive to the effects of NF_3 upon germination. Squash seeds and sudan grass seeds were inhibited almost completely in their ability to germinate after exposure of NF_3 for a period of 6 to 8 hours. However, 50% inhibition of these seeds was achieved by exposure periods of less than one hour. The fluoride content of seeds exposed to NF_3 prior to germination was determined (Table V). While the fluoride content was increased by exposure to NF_3, those seeds which germinated to an extent of 50% after exposure had a fluoride content which was less than the content of seeds germinated in the presence of either sodium fluoride or hydrogen fluoride. From the data presented, it could be concluded that exposure of seeds to atmospheres of NF_3 may result in effects upon germination which are not entirely due to fluoride ion alone. However, since there is a very small difference in the fluoride content of seeds exposed to any of these agents the major effect could be the formation or presence of fluoride ion in the seed during its germination processes. Experiments in which the seeds were washed after an NF_3 exposure and prior to germination were carried out (Table V). It can be seen that an increase had occurred in the number of seed germinating upon washing after an NF_3 exposure, except with sudan grass and pea. For these species (comparing 0.8 and 6.5 hr exposures respectively), a decrease or no change was observed. This suggests that a residual fluoride ion may be present on the seed coat after exposure to NF_3. We assume that fluoride ion is inhibiting the germination of NF_3 exposed seeds since the fluoride content of exposed seeds is reduced by washing before germination.

TABLE V

Effect of Exposure of Seeds to Nitrogen Trifluoride on Seed Germination

Plant Species	Exposure Time in Hours to 100% NF_3 Atmosphere	Percent Germination	Fluoride Content After Germination µg/g Dry Wt
Bean			
unwashed	0	89	4
unwashed	1	83	29
unwashed	6	51	32
unwashed	8	20	82
washed*	6	70	45
Corn			
unwashed	0	98	2
unwashed	1	80	13
unwashed	2.25	26	137
unwashed	8	0	297
washed	2.25	75	25
Squash			
unwashed	0	69	3
unwashed	0.7	45	38
unwashed	1	7	406
unwashed	8	0	1,500
washed	0.7	82	53
Sudan Grass			
unwashed	0	63	2
unwashed	0.8	14	178
unwashed	1	24	25
unwashed	8	0	682
washed	0.8	7	152
Pea			
unwashed	0	87	4
unwashed	1	78	11
unwashed	6.5	89	15
unwashed	8	31	53
washed	6.5	87	10

*All seeds exposed to NF_3 were germinated immediately after exposure. A slight delay was necessary when exposed seeds were washed prior to germination. Experiments with washed seeds consisted of washing the exposed seeds in fluoride-free distilled water five times before germinating.

Effect of Exposure of Seeds to Tetrafluorohydrazine on Germination

Preliminary experiments have been completed on the effect of N_2F_4 exposure on the germination of dry seeds. Exposure of seeds to N_2F_4 in air at 1,000 and 10,000 ppm for 1 hour was found to have essentially no effect upon subsequent germination of the exposed seeds. One hour exposure of seeds to 100,000 ppm (in air) and undiluted N_2F_4 completely inhibited germination of all 5 seed types. Further experiments will establish more closely the level of N_2F_4 exposure necessary to give a graded effect of this agent on seed germination.

Effect of Exposure of Seeds to Bromine Pentafluoride on their Germination

The exposure of dry seeds to 100 and 250 ppm of bromine pentafluoride in dry air caused a drastic inhibition of germination of seeds tested (Table VI). Squash and sudan grass seeds were the most sensitive to BrF_5 effects with corn following closely. Bean and pea were somewhat more resistant to BrF_5 effects than the other seeds. The fluoride content after germination illustrates the parallel of increased fluoride content to increased inhibition of germination. Squash showed a remarkably high fluoride content and appeared to be the most sensitive to BrF_5 vapor.

TABLE VI

Effect of Bromine Pentafluoride on Seed Germination

Plant Species	Percent Germination After One Hour Exposure to BrF_5 At			Fluoride Content After Germination μg/g Dry Wt		
	Control	100 ppm	250 ppm	Control	100 ppm	250 ppm
Bean	87	33	22	5	144	102
Corn	99	2	2	5	308	320
Pea	94	26	7	8	158	106
Squash	74	0	0	11	1,368	1,478
Sudan Grass	71	0	1	16	409	471

Effect of Chlorine Trifluoride on Seed Germination

The exposure of seeds to atmospheres of ClF_3 in air resulted in a decreased germination of all seeds (Table VII). While the effects were not entirely reproducible, it is evident that the effects of ClF_3 are similar to those of BrF_5. Again, sudan grass, squash, and corn seeds were the most sensitive to atmospheres of ClF_3 in air. The fluoride content of the ClF_3 treated seeds was not measured. However, the same order of relative sensitivity of seeds to similar concentrations of ClF_3 in air as BrF_5 in air suggests that similar levels of fluoride are present in the ClF_3-exposed seeds.

TABLE VII

Effect of Chlorine Trifluoride on Seed Germination

	Percent Germination After One Hour Exposure to ClF_3						
Concentration ClF_3 in Air (ppm)	0	50	55	100	100	130	900
Plant Species							
Bean	87	91	65	82	76	0	0
Corn	99	87	28	71	17	0	0
Pea	94	94	35	78	32	0	0
Squash	74	23	3	4	0	0	0
Sudan Grass	71	34	6	7	4	0	0

Exposure of Plant Seedlings to Chlorine Trifluoride in Air

The exposure of plant seedlings to gaseous ClF_3 in air at 500 and 2,000 ppm by volume for 5 minutes resulted in extensive damage to the majority of the plants (Tables VIII through XI). ClF_3 at 500 ppm caused extensive and immediate damage to the leaves and stems of all plants exposed except corn, where damage is reflected in a substantial height reduction (Table VIII). Fluoride contents varied widely between species of plants. Corn and pea plants were much lower in fluoride content after exposure to 500 ppm ClF_3 than bean, squash and sudan grass. The lower fluoride content may be related to the nature of leaf surfaces, especially their wax or hydrocarbon coating. In all exposures of plants, the ClF_3 atmosphere rapidly decomposed when the plants were placed into the exposure chamber. Thus, it appears that during the brief exposures the plants were rapidly scavenging the ClF_3 in a manner observed with BrF_5 exposures of plants.

Exposure of the young plants to ClF_3 atmospheres of 2,000 ppm in air for 5 minutes resulted in more extensive damage to all plant species than observed with the 500 ppm exposures. Exposure to the higher concentration of ClF_3 caused a nearly complete destruction of exposed tissues of all species except corn. Very high levels of fluoride were found in all of the exposed seedlings. However, 7 days after exposure, corn and pea plants had greatly reduced their fluoride content while bean and sudan grass retained very high levels of fluoride.

The extensive destruction of plant tissues by ClF_3 appears to occur largely at the exposed surfaces of the plants. The tissues which are protected, such as the coleoptile of the corn seedlings, appear to remain visible and show sign of continuing growth.

Exposure of Seedlings to N_2F_4 in Air

After several preliminary experiments, two concentrations of N_2F_4 in air were selected for exposure of plant seedlings. N_2F_4 at 1,000 ppm for 30 minutes had only a slight effect upon plant height and produced only a slight increase in fluoride content (Tables XII and XIII) Sudan grass had showed the greatest decrease in height (31 ± 4 cm for controls and 24 ± 3 cm for the treated plants 7 days after exposure). However, increase in fluoride content was greatest with bean plants (6.6 μg/g fresh wt for control plants and 42.2 μg fluoride/g fresh wt for the treated plants). Visible injury was observed with bean, corn and sudan grass plants at the 1,000 ppm concentration of N_2F_4.

Exposure of plants to N_2F_4 at a concentration of 10,000 ppm in air for 30 minutes reduced growth rates of all plants exposed (Tables XIV and XV). Growth rate of the plants was reduced to a greater extent when the plants

TABLE VIII

Effects of Exposure of 10-day Old Seedlings to Gaseous ClF_3 at a Concentration of 500 ppm in Air for 5 Minutes

Plant Species	Height Before Exposure (cm)	Height 7 Days After Exposure (cm)	Fluoride Content (μg/g Fresh Wt) Unwashed	Washed	7 Days After Exposure
Bean					
Control	22 ± 2	30 ± 1			6.2
Treated	22 ± 2	6 ± 1	1,345	1,030	1,367
Corn					
Control	15 ± 2	35 ± 3			12.5
Treated	15 ± 2	15 ± 4	238	135	138
Squash					
Control	8 ± 1	20 ± 1			4.0
Treated	8 ± 1	4 ± 2	812	898	1,774
Sudan Grass					
Control	8 ± 1	19 ± 2			6.2
Treated	8 ± 1	5 ± 1	1,044	374	603
Pea					
Control	10 ± 1	27 ± 1			3.3
Treated	10 ± 1	6 ± 1	129	121	95

TABLE IX

Injuring Effects of Exposure of 10-day Old Seedlings to Gaseous ClF_3 at a Concentration of 500 ppm for 5 Minutes

Plant Species	Immediately After Exposure	One Day After Exposure	Seven Days After Exposure
Bean	Leaves wilted, curled edges, light brown-green, stem turgid from cotyledons down, but limp above that point.	Necrosis from primary leaves to cotyledons, stem green.	Stem viable.
Corn	Leaf tips necrotic, leaf was mottled green and orange-brown (discolored spots less than 25% of area).	Leaves wilted, orange-brown spots now gray-green.	Compared with controls living portions of leaves lighter green.
Squash	75% of area of primary leaves and cotyledons brownish, stem wilted.	Cotyledons and primary leaves dehydrated and gray-green. Stem turgid.	Growing tips necrotic.
Sudan Grass	Leaf tips and edges brown, some of leaves had mottled appearance of corn.	Leaves wilted, stem unaffected.	Leaf portions not protected by coleoptile dry and brown.
Pea	1/4 of leaves had orange-brown spots, plant slightly wilted.	Stem turgid and green about 4 cm above ground; beyond, leaves and stem wilted.	Original growth dry and brown. New growth green and turgid.

TABLE X

Effects of Exposure of 10-day Old Seedlings to Gaseous ClF_3 at a Concentration of 2,000 ppm in Air for 5 Minutes

Plant Species	Height Before Exposure (cm)	Height 7 Days After Exposure (cm)	Fluoride Content (μg/g Fresh Wt) Unwashed	Washed	7 Days After Exposure
Bean					
Control	24 ± 3	33 ± 2			11.6
Treated	24 ± 3	2	2,423	2,787	1,907
Corn					
Control	12 ± 2	29 ± 5			4.2
Treated	12 ± 2	17 ± 4	1,580	607	383
Squash					
Control	8 ± 1	19 ± 2			3.2
Treated	8 ± 1	-	2,351	1,226	-*
Sudan Grass					
Control	10 ± 1	21 ± 3			68.4
Treated	10 ± 1	-	4,718	2,518	8,598
Pea					
Control	10 ± 1	26 ± 2			15.0
Treated	10 ± 1	-	2,031	1,347	291

* Sample lost during analysis

- Plants overly damaged and not measurable

TABLE XI

Injuring Effects of Exposure of 10-day Old Seedlings to Gaseous ClF_3 at a Concentration of 2,000 ppm for 5 Minutes

Plant Species	Immediately After Exposure	Two Days After Exposure	Seven Days After Exposure
Bean	Stems less turgid, leaves wilted and brown.	Complete plant necrotic with exception of 1 to 2 cm of stem which was turgid and green.	Same appearance.
Corn	Leaves and coleoptile yellow-brown and wilted. Leaf tips, edges and perhaps central vein necrotic.	1/3 of plants dead, remaining 2/3 had green leaves with necrotic tips.	4 of 15 plants dead. 11 had necrotic tips and edges extending from 5 to 10 cm down leaf, plants less turgid, leaves appearing after exposure normal. Portions of leaves protected by the coleoptile were not visibly affected. Because of this protection, the plant continued to grow after exposure. This is in contrast to beans where the growing tip was unprotected and the plant died.
Squash	Leaves and stem wilted, cotyledons dark brown-green, primary leaves light brown-green on edges.	1 cm of living tissue visible above ground.	No living tissue visible. One seed germinated after exposure.
Sudan Grass	Plants light yellow-brown and limp. Seem more affected than corn.	Plants dehydrated and brown-green.	10% of plants had green tissue, remaining were completely brown and dry.
Pea	5 to 6 cm still turgid, upper 5 to 6 cm brown and limp as was 75 to 100% of leaf area.	1 cm of green living stem.	7 of 13 plants had shoots 3 cm long, remainder of plants and 6 other plants dry and brown with exception of 1 to 2 cm of stem.

TABLE XII

Effects of Exposure of 10-day Old Seedlings to Gaseous N_2F_4 at a Concentration of 1,000 ppm in Air for 30 Minutes

Plant Species	Height Before Exposure (cm)		Height Seven Days After Exposure (cm)		(μg F^-/g fresh wt.) F^- Content After Exposure			F^- Content Seven Days After Exposure	
					Unwashed		Washed		
	Control	Treated	Control	Treated	Control	Treated	Treated	Control	Treated
Bean	20 ± 2	20 ± 2	24 ± 1	24 ± 4	6.6	42.2	20.6	5.1	23.3
Corn	23 ± 2	23 ± 2	40 ± 2	40 ± 5	5.0	18.9	8.8	4.7	4.0
Squash	9 ± 1	9 ± 1	14 ± 2	16 ± 2	1.0	8.3	4.4		3.2
Sudan Grass	17 ± 3	17 ± 3	31 ± 4	24 ± 3	4.6	10.6	9.9	1.2	4.3
Pea	9 ± 1	9 ± 1	17 ± 2	14 ± 2		9.2	5.7	5.2	5.0

TABLE XIII

Injuring Effects of Exposure of 10-day Old Seedlings to Gaseous N_2F_4 at a Concentration of 1,000 ppm in Air for 30 Minutes

Plant Species	Immediately After Exposure	One-Two Days After Exposure	Seven Days After Exposure
Bean	None	From less than 25 to more than 75% of leaf area necrotic.	Necrotic area still apparent, but subsequent growth showed no effect.
Corn	None	None	Leaf tip and edges of ≈1/8 of plants had light green spots.
Squash	None	None	None
Sudan Grass	None	None	Up to 4 cm of leaf tip on all plants necrotic.
Pea	None	None	None

TABLE XIV

Effects of Exposure of 10-day Old Seedlings to Gaseous N_2F_4 at a Concentration of 10,000 ppm in air for 30 minutes

Plant Species		Height Before Exposure (cm)		Height Seven Days After Exposure (cm)		(μgF^-/g fresh wt.) F^- Content After Exposure			F^- Content Seven Days After Exposure	
						Unwashed		Washed		
		Control	Treated	Control	Treated	Control	Treated	Treated	Control	Treated
*I.	Bean	17 ± 1	17 ± 1	22 ± 1	18 ± 1	6.3	47.9	66.1	5.3	20.1
**II.	Bean	23 ± 2	23 ± 2	31 ± 2	19 ± 4		66.7	39.8	7.8	22.49
I.	Corn	21 ± 2	21 ± 2	35 ± 3	21 ± 2	4.3	20.7	17.0	5.9	18.9
II.	Corn	14 ± 2	14 ± 2	34 ± 4	7 ± 2		29.3	21.9	3.1	40.1
I.	Squash	7 ± 2	7 ± 2	21 ± 2	9 ± 2	12.8	14.0	9.4		9.1
II.	Squash	8 ± 2	8 ± 2	21 ± 2	14 ± 2		33.7	39.4	4.0	36.3
I.	Sudan Grass	14 ± 2	14 ± 2	16 ± 2	14 ± 2	14.7	25.5	12.7		66.8
II.	Sudan Grass	7 ± 1	7 ± 1	16 ± 2	4 ± 1		268.7	97.6	7.1	69.7
I.	Pea	12 ± 1	12 ± 1	19 ± 2	13 ± 2	3.0	39.7	36.9	3.7	18.4
II.	Pea	9 ± 1	9 ± 1	24 ± 1	11 ± 2		25.2	22.0	2.1	35.6

*I. Exposed in small chamber.
**II. Exposed in large chamber.

TABLE XV

Injuring Effects of Exposure of 10-day Old Seedlings to Gaseous N_2F_4 at a Concentration of 10,000 ppm in Air for 30 Minutes

Plant Species	Immediately After Exposure	Three Days After Exposure	Seven Days After Exposure
I. Bean	None		Small brown spots on leaves, edges curled, growing tip necrotic, although stem still turgid. 2 plants were dead.
II. Bean	4 hours after exposure, 75 to 100% of leaf area necrotic and leaf edges dry, inner area spotted brown.	≈100% of leaf area necrotic 3 of the 10 plants had viable growing tips.	Original primary leaves necrotic on all plants; however, each plant had new growth.
I. Corn	None		All leaf tips brown from 5 to 15 cm.
II. Corn	4 hours after exposure from 50 to 100% of leaf area was necrotic.	More than 75% of all leaf area was necrotic.	The necrotic area was pale brown and no sign of recovery was visible, as it was in II Bean.
I. Squash	None		Edges of primary leaves curled and yellow, cotyledons also having a yellow border.
II. Squash	Necrotic area on cotyledons around edges extending towards center, primary leaves unaffected.	Of the 5 species tested. squash showed best resistance to N_2F_4. 25 to 50% of cotyledon area necrotic, but no affect on primary leaves.	≈1/2 primary leaves with slightly curled edges, but growing tip unaffected.

TABLE XV (Concluded)

Plant Species	Immediately After Exposure	Three Days After Exposure	Seven Days After Exposure
I. Sudan Grass	None		2/3 plants dead, remaining 1/3 have brown edges.
II. Sudan Grass	50 to 100% leaf area necrotic.		Necrotic area went up to 75%.
I. Pea	None		Exposed leaves necrotic, those growing after exposure unaffected.
II. Pea	4 hours after exposure 25 to 50% of leaf area was necrotic.		Edges curled, 25% of leaf area dark green.

were exposed to N_2F_4 in the large chamber than when exposed in the small chamber, possibly the result of a scavenging effect by the plants for N_2F_4. Also, the decomposition of N_2F_4 to nitrosyl fluoride (NOF) and nitrogen dioxide (NO_2) has been found to occur at a more rapid rate in the large chamber due to the increased residence time of atmosphere in the chamber.

Fluoride content of plants exposed to 10,000 ppm increased over that of plants exposed to 1,000 ppm for the same length of time (30 minutes). The fluoride content of the plants exposed to N_2F_4 is very low, however, compared to the levels of fluoride found in plants exposed to ClF_3, even though damage to the plants by N_2F_4 is very extensive. From the injury effects observed and the relatively low levels of fluoride present, the effects of N_2F_4 on plants may be partially a systemic effect which is not dependent upon the amount of fluoride present after exposure. However, if a comparison is made with the fluoride levels from NF_3, HF or NaF^- exposed plants or seeds, the fluoride levels are very comparable.

Effect of Irrigation of Plants with Inorganic Fluoride-Water Reaction Solutions

Attempts to prepare solutions of NF_3 in water resulted in solutions of very low fluoride content since the reaction of NF_3 with water is negligible. These solutions did not have an observable effect on plants. The reaction of gaseous N_2F_4 in water was also found to be very slow. Solutions of N_2F_4 in water had 9 μg or less of fluoride in 1 ml as determined by fluoride analysis. The only observable effect of N_2F_4 solutions on the plants treated was leaf curling effect on squash. Bean, corn and sudan grass plants did not appear to be affected by the solution.

The reactions of gaseous ClF_3 and BrF_5 with water were extremely vigorous. Small flames or flashes of light were observed at the point of contact between the water and gaseous BrF_5. Fluoride contents of the solutions resulting from these reactions were in agreement with values based upon the measured flow rate of the ClF_3 and the BrF_5 gases into the water contained in a polyethylene reaction bottle.

The observable effects on plants irrigated with the reaction mixture of ClF_3 and H_2O diluted to contain 50 and 100 ppm of fluoride are listed in Tables XVI and XVIII. Bean plants appeared to be considerably more susceptible to ClF_3-H_2O reactions solutions than the other plants examined. While plant height was not affected during the 3-week observation period, necrotic damage was evident, but no significant change was found in the fluoride content of the leaves and stems of these plants when compared with control plants. Fluoride levels ranged from 10 to 109 μg/gm dry weight of tissue depending on the plant species.

A reaction mixture of BrF_5 and water containing 470 ppm of fluoride was found to affect only the pea, squash and sudan grass plants within

TABLE XVI

Effects of Watering Plants with ClF_3-H_2O Reaction Mixture Containing 50 ppm F^-*

Days After First Watering	Bean	Corn	Pea	Squash	Sudan Grass
3	1/2 of plants had pale green and brown spotted leaves; control had dark green leaves.	No effect.	No effect.	No effect on leaves. The cotyledons were spotted light and dark green.	No effect.
18		The plants appeared vigorous and showed continued growth.	Leaves on lower 1/3 of the stem were necrotic and the top 2/3 of the leaves plus the stem showed no effects.	1/2 of the leaf area of the cotyledons was necrotic.	The plants appeared vigorous and showed continued growth.

* The 10-day old plants were watered for 2 consecutive days with ClF_3-H_2O reaction solutions which contained 50 ppm fluoride.

TABLE XVII

Effects of Watering Plants with ClF_3-H_2O Reaction Mixture Containing 100 ppm F^-*

Days After First Watering	Bean	Corn	Pea	Squash	Sudan Grass
4	Leaves were pale green with necrotic spots.	Leaves were pale green with definite yellow stripes.	Peas appeared healthiest with 3 of the 11 plants having curled leaves.	Squash cotyledon had necrotic spots on leaf edge. No effect visible on the leaves.	Leaves spotted.
7	Leaves of all plants were mottled green and brown. 1/3 of the plants lost the original leaves. The growing tip was brownish-green.	Leaves exhibit a brown and green striping. 1/4 of the leaves had a dry border and tip.	Lower leaves of all plants have small necrotic areas. Leaves at the top of the plant show no effects.	Edge of cotyledons was necrotic. The leaves showed some curling and a mottled appearance.	1/2 of the leaves are reddish-brown spotted.
18	All original leaves were lost and the growing point completely destroyed. However, further down the plant, new growth had started.	Plants appeared vigorous and continued growth.	Leaves on the lower 1/3 of the stem were necrotic, but the remainder appeared healthy.	Although the cotyledons were necrotic, the leaves had only a slight discoloration.	Plants appeared vigorous and continued growth.

* The 10-day old plants were watered for 2 consecutive days with ClF_3-H_2O reaction solutions which contained 100 ppm of fluoride.

TABLE XVIII

Effects of Watering Plants with BrF_5-H_2O Reaction Mixture Containing 470 ppm F^-*

Days After First Watering	Bean	Corn	Pea	Squash	Sudan Grass
14	No effects.	No effects.	On 2/3 of the light plants, the leaves were brown and dry. The remaining leaves were light green with some necrotic spots.	All cotyledons were a yellow-green. The primary leaves were normal.	The leaves were yellow-green with distinct dark green stripes.

* The 10-day old plants were watered for 2 consecutive days with the BrF_5-H_2O reaction which contained 470 ppm fluoride.

a 2-week observation period (Table XVIII). These effects were slight since growth heights were not affected. Also, the fluoride content of the leaves and stems of the treated plants did not change significantly from the control plants. Fluoride contents ranged from 13 to 88 mg/gm dry weight depending on the plant species.

The results obtained thus far indicate that BrF_5 and ClF_3 react with water to yield mixtures which, when administered to the soil, can affect plants growing in the treated soil without an appreciable change in the fluoride contents of the stems or leaves of those plants.

SECTION VIII

DISCUSSION AND CONCLUSIONS

Plants vary to a considerable degree in their susceptibility to fluorine-containing compounds such as hydrogen fluoride and fluorosilicic acid (H_2SiF_6). Chlorotic and necrotic markings, uptake of fluoride, as well as growth and yield of plants are known to vary with both plant species and plant varieties within a species (ref. 75, 76, 77).

Notable observations thus far on the effects of inorganic fluoride oxidizing agents on plants include the extreme leaf destruction and rapid accumulation of fluoride during short exposures to air containing the interhalogens, ClF_3 and BrF_5. The plants appeared to scrub the interhalogens from the atmosphere and retain large amounts of fluoride. Plants exposed to NF_3 and N_2F_4 exhibited no signs of leaf damage. More protected areas, for example, the unexposed growing tip of corn plants generally survived exposure to the concentrations of ClF_3 and BrF_5 in air, which caused extensive leaf damage. Exposure of plants to atmospheres of NF_3, N_2F_4, ClF_3 or BrF_5 did not appear to produce appreciable systemic effects. Effects observed were mostly at the exposed surfaces of the plant.

Exposure of seeds to solutions of sodium fluoride and hydrogen fluoride during germination was used as a guide to establish the nature of the inhibiting effects of exposure of seeds to the inorganic fluoride oxidizing agents. The fluoride content of seeds exposed to gaseous inorganic fluoride-air atmospheres could be correlated with inhibition of germination. Whether in every case inhibition of germination resulted from the accumulation of fluoride is doubtful. For example, squash seeds were found to accumulate much greater amounts of fluoride from a BrF_5-air atmosphere than other seeds. These results could indicate that BrF_5 may react with squash seeds in a different manner than with the other seeds tested.

Further tests would be needed to differentiate the effects of inorganic fluorides on seed germination.

Plants were damaged when they were irrigated with solutions formed by the reaction of ClF_3 and BrF_5 with water. These effects appeared to differ between ClF_3 and BrF_5, since ClF_3-H_2O reaction mixture containing 50-100 ppm of fluoride affected bean plants and yet solutions of 470 ppm of fluoride derived from BrF_5 did not. The other plants also showed some differences in their response to ClF_3-H_2O and BrF_5-H_2O reaction mixtures.

Neither germination, seedlings, nor mature plants appear to be seriously affected by low concentrations of NF_3 or N_2F_4 in air. In contrast, even very low concentrations of ClF_3 or BrF_5 in air are very damaging. The damage appears to result from a rapid reaction between the interhalogens and the plant or seed surfaces.

Table XIX is a listing of plant injury indices for inorganic fluoride atmospheres. Except for the BrF_5 experiments, the concentrations shown were those found to be graded responses by the plants to the specified atmospheres.

TABLE XIX

Injury Indices for NF_3, N_2F_4, and ClF_3 in Air*

Type of Exposure	Plant Species				
	Bean	Corn	Squash	Sudan Grass	Pea
ClF_3-500 ppm for 5 min	4	2	4	4	3
ClF_3-2,000 ppm for 5 min	5	3	5	5	5
BrF_5-10,000 ppm for 30 min	5	5	5	5	5
N_2F_4-1,000 ppm for 30 min	1	1	0	1	0
N_2F_4-10,000 ppm for 30 min	3	4	2	4	2
NF_3-100 ppm for 60 min	0	1	0	0	0
NF_3-10,000 ppm for 60 min	0	2	2	1	0

* The plant injury index is based on a 0 to 5 scale with 0 as no injury and 5 as death.

APPENDIX I

ADDITIONAL PHYSICAL PROPERTIES OF INORGANIC FLUORIDES

ADDITIONAL PHYSICAL PROPERTIES OF INORGANIC FLUORIDES

Compound	Heat of Formation	Heat of Fusion	Heat of Vaporization	Vapor Density	Vapor Pressure Equation Constant	Trouton's Constant	References
Nitrogen Trifluoride	29.7±1.8		2,769		a. 6.77 b. -- c. $\frac{501.93}{(T-15.37)}$	19.21	2, 3
Tetrafluoro-hydrazine	2.0±2.5		3,170		a. 6.33 b. -692 c. --	15.9	9
Chlorine Trifluoride		1,819.3 cal/mole	6,580	at 0°C 0.0027 gm/cc at 12°C 0.0040 40°C 0.0105		23.7	24, 25, 26
Bromine Pentafluoride		1,740 cal/mole	7,200 at b.p.			23.7	24, 32
Oxygen Difluoride	7.6±2.0 at 298.15°K		2,650 at -144.8°C	at 0°C 0.0023 gm/cc 21°C 0.0021 g/cc		20.65	

APPENDIX II

SEED GERMINATION CRITERIA

The following criteria for seed germination are taken from U.S. Department of Agriculture Handbook No. 30, "Testing Agricultural and Vegetable Seeds," (ref.135).

A. Bean

Normal

1. The seedling must have two primary leaves, or at least one primary leaf, even though one or both cotyledons are absent. The terminal bud must be present in either case.

2. The seedling must have a primary root or a set of adventitious or secondary roots sufficient to anchor it when grown in soil or sand, provided the hypocotyl is not badly shortened.

3. The normal seedling must have a fairly well developed hypocotyl with no prominent breaks or deep lesions. Healed breaks are to be considered as normal provided the seedling is not spindly.

4. Normal seedlings may include those with slight infection from fungi, provided the essential structures have not been seriously damaged and appear able to carry on their normal functions at the time of evaluation. If a few seedlings with total or partial decay of the plumule are found, they may be counted as normal, provided the hypocotyl and root are well developed.

5. Spirally twisted and curled root-hypocotyl held within the rough seed coat, causing delayed development; otherwise normal, count such seedlings as normal.

Abnormal

1. No primary leaves or terminal bud.

2. No primary leaves, but with a terminal bud.

3. No primary leaves, but terminal bud present and axillary buds in one or both of the cotyledons.

4. A malformed hypocotyl which may be characterized by open splits, or appear curled, shortened, or thickened.

5. No primary roots or well-developed set of adventitious or secondary roots.

6. Various combinations of the above-named abnormal types.

B. Corn

Normal

1. One primary root, usually with secondary roots present.

2. No primary roots, but with at least two vigorous secondary roots, provided the grain is not badly decayed, and the shoot is well-developed.

3. Well-developed green leaves, usually broken through the coleoptile by the end of the test period.

4. Twisted and curled shoots bound by the tough seed coat, provided the shoot is not decayed.

5. Slight infection by fungi provided none of the essential seedling structures have been damaged.

Abnormal

1. No primary or secondary roots.

2. No primary roots, but small, weak secondary roots.

3. No plumule, but only the white sheath or coleoptile.

4. A shortened plumule extending no more than one-half the way up through the coleoptile.

5. A thickened and shortened shoot; often the result of overtreated seed with chemicals.

6. A spindly and pale shoot usually associated with moldy seeds.

7. Albino seedlings, which will not develop into plants because of lack of chlorophyll.

8. Shattered or longitudinally split leaves, with or without splitting of the coleoptile.

9. Decayed shoots.

10. Various combinations of the above-named abnormal types.

C. Squash

Normal

1. A well-developed primary root with or without secondary roots.

2. A stubby primary root with at least two strong and vigorous adventitious roots, providing the hypocotyl is not shortened very much.

3. A long, well-developed hypocotyl.

4. Two intact cotyledons.

5. Slight infection by fungi, provided none of the essential seedling structures have been damaged.

Abnormal

1. No primary root, a stubby primary root, or a stubby primary root weak secondary roots which are usually associated with a short hypocotyl.

2. A malformed hypocotyl which may be shortened or thickened.

3. Thickened and shortened hypocotyles and roots owing to injury from chemical treatment.

4. Decayed cotyledons or other essential seedling structures, provided the decay was not the result of improper test conditions.

5. Various combinations of the above-named abnormal types.

D. Sudan Grass

Normal

1. One primary root.

2. Well-developed, green leaves, usually broken through the coleoptile by the end of the test period.

3. Slight infection by fungi, provided none of the essential seedling structures have been damaged.

4. Red coloration on the roots and on the coleoptile of the shoot.

Abnormal

1. No roots.

2. A weak, spindly, and usually shortened primary root, which is often associated with decay of the grain.

3. No plumule, but only the white sheath or coleoptile.

4. A shortened plumule, extending no more than one-half the way up through the coleoptile.

5. A spindly and pale plumule, usually associated with moldy seeds.

6. Shattered and longitudinally split plumules, with or without splitting of the coleoptile.

7. Decayed plumules, provided the decay is not the result of improper test conditions.

8. Various combinations of the above-named abnormal types.

E. Pea

Normal

1. A well-formed root, with or without secondary or adventitious development.

2. A strong epicotyl with fairly long stem.

3. A well-developed epicotyl with the leaves and terminal bud intact.

4. The seedling should not be broken away from the cotyledons.

5. A primary root or a set of secondary or adventitious roots.

6. A fairly well-developed stem with no prominent breaks or deep lesions which might interfere with the conducting tissues.

7. A terminal bud with at least one first leaf and an intact growing point.

8. Two shoots, provided the seedling appears vigorous and at least one of the shoots has a normal epicotyl and root.

9. Slight infection by fungi, provided essential seedling parts have not been seriously damaged and appear able to carry on their normal functions at the time of evaluation.

Abnormal

1. No primary root or well-developed secondary or adventitious roots.

2. A malformed stem which may be characterized by severe open splits, and curled, shortened, or thickened development.

3. No epicotyl, or an epicotyl without terminal bud.

4. Two shoots, both of which appear weak and spindly often partially broken away from the cotyledons.

5. Decayed seedlings caused by the spread of decay from the cotyledons of the developing seedlings.

6. Various combinations of the above-named abnormal types.

REFERENCES

1. Ruff, O. and L. Staub. Nitrogen fluorides: Partial diagram of the system: NH_3-HF. Z anorg. allgem. Chem. 212, 399-400 (1933).

2. Hoffman, J. H. and R. G. Neville. Nitrogen fluorides and their organic derivatives. Chem. Reviews 62, 1-18 (1962).

3. Pankratov, A. V. Chemistry of some inorganic nitrogen fluorides. Russ. Chem. Reviews 32, 157-164 (1963).

4. Sheridan, J. and W. Gordy. The nuclear quadrupole moment of N^{14} and the structure of nitrogen trifluoride from microwave spectra. Phys. Rev. 79, 513-515 (1950).

5. Schomaker, V. and Chia-Si Lu. An electron-diffraction investigation of nitrogen trifluoride. J. Am. Chem. Soc. 72, 1182-1185 (1950).

6. Ghosh, S. N., R. Trambarulo and W. Gordy. Electric dipole moments of several molecules from the stark effect. J. Chem. Phys. 21, 308-310 (1953).

7. Kennedy, A. and C. B. Colburn. Strength of the N-F bonds in NF and of N-F and N-N bonds in N_2F_4. J. Chem. Phys. 35, 1892-1893 (1961).

8. Hurst, G. L. and S. I. Khayat. " 'Hydrodysis of the nitrogen fluorides,' Advances in Propellant Chemistry," Advances in Chemistry Services, No. 54, Robert F. Gould (editor). The American Chemical Society, 245-260 (1966).

9. Colburn, C. B. and A. Kennedy. Communications to the editor. "Tetrafluorohydrazine" J. Am. Chem. Soc. 80, 5004 (1958).

10. Colburn, C. B. In International Symposium on Fluorine Chemistry, p.30, University of Birmingham and The Chemical Society, London, 1959.

11. Sales Literature, Air Products, Inc. Allentown, Pennsylvania, 1960.

12. Sales Literature, Stauffer Chemical Company, New York, N.Y., 1960.

13. Lide, D. R. and D. E. Mann. Microwave spectrum and structure of N_2F_4. J. Chem. Phys. 31, 1129-1130 (1959).

14. Johnson, F. A. and C. B. Colburn. The tetrafluorohydrazine-difluoroamino radical equilibrium. J. Am. Chem. Soc. 83, 3043-3047 (1961).

15. Frazer, J. W. Preparation of N, N-difluoromethylamine and N-N-difluoroethylamine. J. Inorg. Nucl. Chem. 16, 63-66 (1960).

16. Frazer, J. W., B. E. Holder and E. F. Worden. The preparation and identification of N-fluoro-N-trifluoromethyldiazine-N'-oxide. J. Inorg. Nucl. Chem. 24, 45-52 (1962).

17. Petry, R. C. and J. P. Freeman. Communications to Editor. Tetrafluorohydrazine: A versatile intermediate for the synthesis of N-fluoro compounds. J. Am. Chem. Soc. 83, 3912 (1961).

18. Beach, L. K. Oxidation of N_2F_4 to NOF and NF_3. J. Inorg. Nucl. Chem. 26, 2033-2034 (1964).

19. Jones, E. A., T. F. Parkinson and R. B. Murray. The infrared and raman spectra of chlorine trifluoride. J. Chem. Phys. 17, 501-502 (1949).

20. Magnuson, D.W. Dielectric constant measurements of chlorine trifluoride at 9400 mc/sec. J. Chem. Phys. 20, 229-232 (1952).

21. Burbank, R. D. and F. N. Bensey. The structures of the interhalogen compounds. I. Chlorine trifluoride at -120°C. J. Chem. Phys. 21, 602-608 (1953).

22. Smith, D. F. The microwave spectrum and structure of chlorine trifluoride. J. Chem. Phys. 21, 609-614 (1953).

23. Meutterties, E. L. and W. D. Phillips. Structure of ClF_3 and exchange studies on some halogen fluorides by nuclear magnetic resonance. J. Am. Chem. Soc. 79, 322-326 (1957).

24. Allied Chemical Company, General Chemical Division. Chlorine trifluoride. Technical Bulletin TA-8532-2, New York, N.Y.

25. Vincent, L. M. and J. Gillardeau. Le trifluorure de chlore. AEC Report No. (CEA) 2360, 1964.

26. Anonymous, Pensalt Chemicals. Chlorine trifluoride, properties and method of handling. Philadelphia, Pa., 1952.

27. Doescher, R. N. The properties of fluorine, oxygen bifluoride and chlorine trifluoride. JPL Project No. TU-21, Contract No. W-04-200-ORD-1482, Memorandum No. 9-16, 1949.

28. Farrar Jr., R. L. Safe handling of chlorine trifluoride and the chemistry of the chlorine oxidases and oxyfluorides. Union Carbide Nuclear Co., AEC Report K-1416, 1960.

29. Ruff, O. and W. Menzel. Bromine pentafluoride. Z. anorg. allgem. Chem. 202, 49-61 (1931).

30. National Bureau of Standards. Selected values of chemical thermodynamic properties. Washington, D.C., 1954.

31. Booth, H. S. and J. T. Pinkston, Jr. The halogen fluorides. Chem. Reviews 41, 421-439 (1947).

32. Brasted, R. C. The halogens. Vol.3. D. Van Nostrand Co., Princeton, N.J., 1954, pp. 209-210.

33. McDowell, R.S. and L. B. Asprey. Infrared spectrum of bromine pentafluoride. J. Chem. Phys. 37, 165-167 (1962).

34. Schnitzlein, J. G. et al. The preparation and purification of OF_2 and determination of its vapor pressure. J. Phys. Chem. 56, 233-234 (1952).

35. Ruff, O. and K. Clusius. Melting points of oxygen and nitrogen trifluoride. Z. anorg. allgem. Chem. 190, 267-269 (1930).

36. Lebeau, P. and A. Damiens. The existence of an oxygen compound of fluorine. Compt. rend. 185, 652-654 (1927).

37. Lebeau, P. and A. Damiens. A new method for the preparation of the fluorine oxides. Compt. rend. 188, 1253-1255 (1929).

38. Brasted, R. C. The halogens. Vol.3. D. Van Nostrand Co., Princeton, N.J., 1954, p. 129.

39. Sutton, L. E. and L. O. Brockway. Electron diffraction investigation of the molecular structure of certain compounds including oxygen fluoride. J. Am. Chem. Soc. 57, 473-483 (1935).

40. Berstein, H. J. and J. Powling. The infrared spectrum of F_2O from 2.5 to 25 μ. J. Chem. Phys. 18, 685-689 (1950).

41. Gmelin's Handbuch der Anorganischen Chemie, Fluor, systemnummer 5. Verlag Chemie, G.M.B.H., Weinheim, Bergstrasse, 1959.

42. Glissman, N. A. and H. J. Schumacher. The spectrum of fluorine oxide. Z. physik. Chem. B24, 328-334 (1934).

43. Ischikawa, F. and T. Takai. Fluorine. III. Stability of oxygen fluoride. Sci. Rpt. Tohoku Univ. 24, 98-106 (1935).

44. Brasted, R. C. The halogens. Vol.3. D. Van Norstrand Co., Princeton, N. J., 1954, pp.130-131.

45. Ruff, O. and W. Menzel. Oxygen fluoride, OF_2. Z.anorg. allgem. Chem. 190, 257-266 (1930).

46. Koblitz, W. and H. J. Schmacher. The thermal decomposition of F_2O. A unimolecular decomposition represented by a reaction of the second order. Z. physik. Chem. B25, 283-300 (1934).

47. Ruff, O. and W. Menzel. Possibility of building higher oxygen fluoride and the properties of oxygen difluoride. Z. anorg. allgem. Chem. 198, 39-52 (1931).

48. Wartenberg, H. V. and G. Klinkott. The heat of formation of fluorine oxide. Z. anorg. allgem. Chem. 193, 409-419 (1930).

49. Streng, A. G. the oxygen fluorides. Chem. Reviews 63, 607-624 (1963).

50. Rhein, R. A. and G. H. Cady. Some reactions of oxygen difluoride. Office of Naval Research, ARPA Order No. 26-60, Task 2, Contract No. Nonr-477 (16).

51. Hu, J. H., D. White and H. L. Johnston. Condensed gas calorimetry No.5, Physical properties of fluorine. J. Am. Chem. Soc. 75, 5642-5645 (1953).

52. Allied Chemical Company, General Chemical Division. Fluorine. Technical Bulletin TA-85411, New York, N.Y., 1961.

53. Cady, G. H. and J. H. Hildebrand. Vapor pressure and critical temperature of fluorine. J. Am. Chem. Soc. 52, 3839-3843 (1930).

54. Brasted, R. C. The halogens. Vol.3. D. Van Nostrand Co., Princeton, N.J., 1954, p.105.

55. Ruff, O. Zur Kenntnis des stickstoff-3-fluorides. Z. anorg. allgem. Chem. 197, 273-286 (1931).

56. Torkelson, T. R., F. Oyen, S. E. Sadek and V. K. Rowe. Preliminary toxicologic studies on nitrogen trifluoride. Toxicol. Applied Pharmacol. 4, 770-781 (1962).

57. Clayton, J. W. The toxicity of fluorocarbons with special reference to chemical constitution. J. Occup. Med. 4, 262-273 (1962).

58. Carson, T. R. and F. T. Wilinski. The acute inhalation toxicity of tetrafluorohydrazine. Toxicol. Appl. Pharmacol. 6, 447-453 (1964).

59. Horn, H. J. Chlorine trifluoride. Army Chemical Center, Md. Report for Contract DA 18-108-CML-4399 (Medical Laboratory Contract Report No.37) 1954.

60. Horn, H. J. Chlorine trifluoride. Army Chemical Center, Md. Progress Report for Hazelton Labs., Falls Church, Va. (Medical Laboratory Contract Report No. 22), 1953.

61. LaBelle, C. W. Studies on the toxicity of oxygen difluoride at levels of exposure from 10 to 0.1 ppm by volume. University of Rochester. Manhattan Project. Pharmacology Report No. 478, 1945.

62. Cianko, G. M. Range find tests on oxygen difluoride. Rocketdyne, Division of North American Aviation, Canoga Park, Calif., 1961.

63. Lester, D. The toxicity of oxygen difluoride. General Chemical Research Laboratory, Allied Chemical Corporation, 1962.

64. Lester, D. and W. R. Adams. The inhalation toxicity of oxygen difluoride. Am. Ind. Hygiene Assoc. J. 26, 562-567 (1966).

65. Machle, W., F. Thamann, K. Kitzmiller and J. Cholak. The effects of the inhalation of hydrogen fluoride. I. The response following exposure to high concentrations. J. Ind. Hyg. 16, 129 (1934).

66. Stokinger, H. E. Toxicity following inhalation of fluoride and hydrogen fluoride in pharmacology and toxicology of uranium compounds. Div. VI, Vol. I, Ed. C. Voegtlin and H. C. Hodge, Mc-Graw-Hill Book Company, New York, N.Y., 1949, p.1084.

67. Rosenholtz, M. J. Pathogenic observations in animals after single, brief exposures to hydrogen fluoride. U.S. Army Chemical Research and Development Labs., Edgewood Arsenal, Md., Technical Report CRDLR 3158, 1962.

68. McClure, F. J. A review of fluorine and its physiologic effects. Physiol. Rev. 13, 277-300 (1933).

69. Hodge, H. C. and F. A. Smith. Fluorine Chemistry Vol. IV. Ed. J. H. Simons. Academic Press, New York, N.Y., 1965, p.1-42.

70. Princi, F. Fluorides: A critical review. III. The effects on man of the absorption of fluoride. Topical Review. J. Occup. Med. 2, 92-99 (1960).

71. Waldbott, G. L. Acute fluoride intoxication. Acta Med. Scand. 174, Suppl. 400, 1-44 (1963).

72. Largent, E. J. Fluorosis. The health Aspects of Fluorine Compounds. Ohio State University Press, 1961.

73. Symposium on the Physiologic and Hygenic Aspects of the Absorption of Inorganic Fluorides. AMA Archives of Industr. Health 21, 303-352 (1960).

74. Hodge, H. C. and F. A. Smith. Fluorine chemistry. Vol. IV. Ed. J. H. Simons. Academic Press, New York, N.Y., 1965.

75. Hitchcock, A.E., P. Y. Zimmerman, and R. R. Coe. The effects of fluorides on milo maize (Sorghum sp.) Contrib. Boyce Thompson Inst. 22, 175-206 (1963).

76. Hitchcock, A. E., P. W. Zimmerman and R. R. Coe. Results of ten year's work (1951-1960) on the effect of fluorides on gladiolus. Contrib. Boyce Thompson Institute 21, 303-344 (1962).

77. McCune, D. C., L. H. Weinstein, J. S. Jacobson and A. E. Hitchcock. Some effects of atmospheric fluorides on plant metabolism. I. Air Poll. Control Assoc. 14, 465-468 (1964).

78. Simonin, P. and A. Pierron. Accessory factors in fluorosis by inhestion of calcium fluoride in the guinea pig. Compt. Rend. Soc. Biol. 124, 669-671 (1937).

79. Leone, N. C. et al. Acute and subacute toxicity studies of sodium fluoride in animals. Public Health Report 71, 459-467 (1956).

80. Lu, F. C. et al. Acute toxicity of sodium fluoride for Rhesus monkeys and other laboratory animals. Acta Pharmacol. Toxicol. 22, 99-106 (1965).

81. Carlson, C. H. et al. Distribution, migration and binding of whole blood fluoride evaluated with radiofluoride. Am. J. Physiol. 199, 187-189 (1960).

82. Call, R. A. et al. Histological and chemical studies in man on effects of fluoride. Public Health Reports 80, 6-10 (1965).

83. Taylor, J. M. et al. Toxic effects of fluoride on the rat kidney I. Acute injury from single large doses. Toxicol. Appl. Pharmacol. 3, 278-289 (1961).

84. Warburg, O. and W. Christian. Isolierung und Kristallisation des Garungsferments Enolase. Biochem. Z. 310, 384-421 (1942).

85. Peters, R. A., M. Shorthouse, and L. R. Murray. Enolase and fluorophosphate. Nature 202, 1331-1332 (1964).

86. Naganna, B., A. Raman, B. Venugopal, and C. E. Sripathi. Potato pyrophosphatases. Biochem. J. 60, 215-223 (1955).

87. Yang, S. F. and G. W. Miller. Biochemical studies on the effect of fluoride on higher plants. 2. The effect of fluoride on sucrose-synthesizing enzymes from higher plants. Biochem. J. 88, 509-516 (1963).

88. Slater, E. C. and W. D. Bonner, Jr. The effect of fluoride on the succinic oxidase system. Biochem. J. 52, 185-196 (1952).

89. Reiner, J. M., K. K. Tsuboi and P. B. Hudson. Acid phosphatase IV. Fluoride inhibition of prostatic acid phosphatase. Arch. Biochem. Biophys. 56, 165-183 (1955).

90. Greenberg, H. and D. Nachmansohn. Studies of acid phosphomonoesterases and their inhibition by diisopropylphosphorofluoridate. J. Biol. Chem. 240, 1639-1646 (1965).

91. Hewitt, E. J. and D. J. D. Nicholas. Cations and anions. Inhibitions and interactions in metabolism and in enzyme activity. Metabolic inhibitors 2. R. M. Hochster and J. H. Quastel, eds., 1963, pp. 311-436.

92. Brennan, E. G., I. A. Leone and R. H. Daines. Fluorine toxicity in tomato as modified by alterations in the nitrogen, calcium, and phosphorous nutrition of the plant. Plant Physiol. 25, 736-747 (1950).

93. Applegate, H. G. and D. F. Adams. Nutritional and water effect on fluoride uptake and respiration of bean seedlings. Phyton (Buenos Aires) 14, 111-120 (1960).

94. Hewitt, E. J. The essential nutrient elements: Requirements and interactions in plants. Plant Physiol. III, F. C. Steward, ed., 137-360 (1963).

95. Wang, C. H. and J. K. Krackov. The catabolic fate of glucose in Bacillus subtilis. J. Biol. Chem. 237, 3614-3622 (1962).

96. White, G. A. and C. H. Wang. The dissimilation of glucose and gluconate by Acetobacter xylinum. 2. Pathway evaluation. Biochem. J. 90, 424-433 (1964).

97. Landau, B. R., G. E. Bartsch, J. Katz, and H. G. Wood. Estimation of pathway contributions to glucose metabolism and the rate of isomerization of hexose-6-phosphate. J. Biol. Chem. 239, 686-696 (1964).

98. Carlson, J. R. and J. W. Suttie. Pentose phosphate pathway enzymes and glucose oxidation in fluoride-fed rats. Am. J. Physiol. 210, 79-83 (1966).

99. Bonner, J. and S. G. Wildman. Enzymatic mechanisms in the respiration of spinach leaves. Arch. Biochem. 10, 497-518 (1946).

100. Laties, G. L. The role of pyruvate in the aerobic respiration of barley roots.. Arch. Biochem. 20, 284-299 (1949).

101. Bonner, J. Biochemical mechanisms in the respiration of the Avena coleoptile. Arch. Biochem. 17, 311-326 (1948).

102. Lustinec, J. and V. Pokorna. Alternation of respiratory pathways during the development of wheat leaf. Biol. Plant. Acad. Sci. Bohemoslov. 4, 101-109 (1962).

103. Pilet, P.-E. Action du fluor et de l'acide beta-indolylacetique sur la respiration des tissus radiculaires. Rev. Gen. Botan. 71, 12-21 (1964).

104. Pilet, P.-E. Action du fluor et de l'acide beta-indolylacetique sur la respiration de disques de feuilles. Bull. Soc. Vaudoise Sci. Nat. 68, 359-360 (1963).

105. Christiansen, G. S. and K. V. Thimann. The metabolism of stem tissue during growth and its inhibition. II. Respiration and ether-soluble material. Arch. Biochem. 26, 248-259 (1950).

106. Ducet, G. and A. J. Rosenberg. Leaf respiration. Ann. Rev. Plant Physiol. 13, 171-200 (1962).

107. McNulty, I. B. and D. W. Newman. Effects of atmospheric fluoride on the respiration rate of bush bean and gladiolus leaves. Plant Physiol. 32, 121-124 (1957).

108. Yang, S. F. and G. W. Miller. Biochemical studies on the effect of fluoride on higher plants. I. Metabolism of carbohydrates, organic acids and amino acids. Biochem. J. 88, 505-509 (1963).

109. Yang, S. F. and G. W. Miller. Biochemical studies on the effect of fluoride on higher plants. 3. The effect of fluorine on dark carbon dioxide fixation. Biochem. J. 88, 517-522 (1963).

110. Hill, A. C., M. R. Pack, L. G. Transtrum and W. S. Winters. Effects of atmospheric fluorides and various types of injury on the respiration of leaf tissue. Plant Physiol. 34, 11-16 (1959).

111. Hill, A. C., L. G. Transtrum, M. R. Pack and W. S. Winters. Air pollution with relation to agronomic crops: VI. An investigation of the "hidden injury" theory of fluoride damage to plants. Agron. J. 50, 562-565 (1958).

112. Thomas, M. D. Air pollution with relation to agronomic crops: I. General status of research on the effects of air pollution on plants. Agron. J. 50, 545-550 (1958).

113. Weinstein, L. H. Effects of atmospheric fluoride on metabolic constituents of tomato and bean leaves. Contrib. Boyce Thompson Inst. 21, 215-231 (1961).

114. McCune, D. C., L. H. Weinstein, J. S. Jacobson, and A. E. Hitchcock. Some effects of atmospheric fluoride on plant metabolism. J. Air Poll. Control Assoc. 14, 465-468 (1964).

115. Adams, D. F. Recognition of the effects of fluorides on vegetation. J. Air Poll. Control Assoc. 13, 360-362 (1963).

116. Applegate, H. G. and D. F. Adams. Effect of atmospheric fluoride on respiration of bush beans. Botan. Gaz. 121, 223-227 (1960).

117. Adams, D. F. and M. T. Emerson. Variations in starch and total polysaccharide content of Pinus ponderosa needles with fluoride fumigation. Plant Physiol. 36, 261-265.(1961).

118. Reckendorfer, P. Ein beitrag zur mikrochemie des rauchschadens durch fluor. Die wanderung des fluors im pflanzlichen gewebe. I. Teil: Die unsichtbaren Schaden. Pflanzen schutz Ber. 9, 33-55 (1952).

119. Tietz, A. and S. Ochoa. Fluorokinase and pyruvic kinase. Arch. Biochem. Biophys. 78, 477-493 (1958).

120. Oelrichs, P. B. and T. McEwan. The toxic principle of Acacia georginae. Queensland J. Agr. Sci. 19, 1-16 (1962).

121. De Oliveira, M. M. Chromatographic isolation of monofluoroacetic acid from Palicourea marcgravii. St. Hil. Experientia 19, 586-586 (1963).

122. McEwan, T. Isolation and Identification of the toxic principle of Gastrolobium grandiflorum. Nature 201, 827-830 (1964).

123. Peters, R. and R. J. Hall. Fluorine compounds in nature; the distribution of carbon-fluorine compounds in some species of Dichapetalum. Nature 187, 573-575 (1960).

124. Peters, R., R. J. Hall, P. F. V. Ward, and M. Sheppard. The chemical nature of the toxic compounds containing fluorine in the seeds of Dichapetalum toxicarium. Biochem. J. 77, 17-23 (1960)

125. Murray, L. R., McConnell, J. D. and Whittem, J.H. Suspected presence of fluoroacetate in Acacia georginae F. M. Bailey Austral. J. Sci. 24, 41-42 (1961).

126. Oelrichs, P. B. and T. McEwan. Isolation of the toxic principle in Acacia georginae. Nature 190, 808-809 (1961).

127. Peters, R. and M. Shorthouse. Fluoride metabolism in plants. Nature 202, 21-22 (1964).

128. Applegate, H. G., D. F. Adams and R. C. Carriker. Effect of aqueous fluoride solutions on respiration of intact bush bean seedlings. I. Inhibition and stimulation of oxygen uptake. Am. J. Botany 47, 339-345 (1960).

129. Peters, R. A., Murray, L.R. and Shorthouse, M. Fluoride metabolism in Acacia geoginae gidyea. Biochem. J. 95, 724-730 (1965).

130 Wade, R. H., J. M. Ross and H. M. Benedict. A method for the detection and isolation of traces of organic fluorine compounds in plants. J. Chromat. 14, 37-45 (1964).

131. Hygienic Guide Series: Fluorine. Prepared by the Industrial Hygiene and Clinical Toxicology Committee of IMA. Am. Md. Hyg. Assoc. 26, 624-627 (1965).

132. Hodge, H. C. and F. A. Smith. Fluorine chemistry Vol. IV. Ed. J. H. Simons. Academic Press, New York, N.Y., 1965, pp. 37-42.

133. Wagner, W. D., B. R. Duncan, P. G. Wright and H. E. Stokinger. Experimental Study of Threshold Limit of NO_2. Arch. Environ. Health 10,455-466(1965).

134. Dost, F. N., D. J. Reed, and C. H. Wang. Exposures of biological systems to inorganic fluoride oxidizing agents. Vol. I. Handling and exposure techniques. Aerospace Medical Research Laboratories Report AMRL-TR-65-223-Vol. I, 1965.

135. U. S. Department of Agriculture. Testing Agricultural and Vegetable Seeds. Agriculture Handbook No. 30 Washington D.C. 1952.

136. Mitchell, J. J. U.S. Department of Agriculture. Inorganic Salt Solutions. Agriculture Handbook No. 126, Washington, D.C., 1958, p.66.

137. Weinstein, L. H., R. H. Mandl, D. C. McCune, J. S. Jacobsen, and A. E. Hitchcock. A semi-automated method for the determination of fluorine in air and plant tissues. Contrib. Boyce Thompson Inst. 22, 207-220 (1963).

138. Reed, D. J., F. N. Dost and C. H. Wang. Exposures of biological systems to inorganic fluoride oxidizing agents. Vol. II. Fluoride analysis by chemical methods. Aerospace Medical Research Laboratories Report AMRL-TR-65-223-Vol. II, 1965.

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Radiation Center Oregon State University Corvallis, Oregon 97331	UNCLASSIFIED
	2b. GROUP N/A

3. REPORT TITLE

INORGANIC FLUORIDE PROPELLANT OXIDIZERS, VOLUME I. THEIR EFFECTS UPON SEED GERMINATION AND PLANT GROWTH

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Interim Report, 15 May 1964 - 15 May 1966

5. AUTHOR(S) (First name, middle initial, last name)

Donald J. Reed, PhD
Frank N. Dost, DVM
Chih H. Wang, PhD

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
November 1967	80	138

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
AF 33(615)-1767	
b. PROJECT NO. 6302	
c. Task No. 630204	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	AMRL-TR-66-187 (VOL I)

10. DISTRIBUTION STATEMENT

Distribution of this document is unlimited. It may be released to the Clearinghouse, Department of Commerce, for sale to the general public.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Aerospace Medical Research Laboratories Aerospace Medical Div., Air Force Systems Command, Wright-Patterson AFB, O. 45433

13. ABSTRACT

Certain inorganic fluorides have been reviewed for their chemical, physical and toxicological properties. The compounds are nitrogen trifluoride; tetrafluorohydrazine; chlorine trifluoride; bromine pentafluoride; oxygen difluoride; hydrogen fluoride, and fluorine. Seeds and seedlings of bean, corn, pea, squash and sudan grass were exposed to air or water mixture of these compounds. Exposure of dry seeds to a gaseous 100% NF_3 atmosphere for 1 to 8 hours caused inhibition of subsequent germination depending on the seed species. Exposure of seeds to a 10% N_2F_4 atmosphere for 1 hour completely inhibited germination of all five species of seeds. Exposure of dry seeds to less than 500 ppm of either bromine pentafluoride or chlorine trifluoride in air drastically reduced their subsequent germination even when the exposure time was for less than one hour. Exposures of plant seedlings to gaseous ClF_3 in air at 500 and 2,000 ppm for 5 minutes resulted in extensive destruction of the plants; bromine pentafluoride-air mixtures were even more damaging than ClF_3 atmospheres. Concentrations of N_2F_4 or NF_3 up to 10,000 ppm in air were required to cause visible damage of plant seedlings after a 30 to 60 minute exposure period. Plant injury was caused by irrigation of plant seedlings with solutions formed by the reaction of ClF_3 or BrF_5 with water. The exposed seeds and seedlings were analyzed for fluoride content. The equipment and methods used for these exposures is described.

DD FORM 1 NOV 65 1473

Security Classification

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Nitrogen trifluoride, NF_3						
Tetrafluorohydrazine, N_2F_4						
Chlorine trifluoride, ClF_3						
Bromine pentafluoride, BrF_5						
Oxygen Difluoride, OF_2						
Hydrogen fluoride, HF						
Fluorine, F_2						
Review of						
chemical properties						
physical properties						
toxicity						
inorganic fluorine propellant oxidizers						
effects on plants						
effects on seed germination						

Security Classification